DRAFT

Water Quality Criteria Report for Simazine

Phase III: Application of the pesticide water quality criteria methodology



Prepared for the Central Valley Regional Water Quality Control Board

Julie C. Bower, Ph.D. and Ronald S. Tjeerdema, Ph.D.

Department of Environmental Toxicology University of California, Davis

June 2016

Disclaimer

Funding for this project was provided by the California Regional Water Quality Control Board, Central Valley Region (CRWQCB-CVR). The contents of this document do not necessarily reflect the views and policies of the CRWQCB-CVR, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

DRAFT

Water Quality Criteria Report for Simazine

Phase III: Application of Pesticide Water Quality Criteria Methodology

Report Prepared for the Central Valley Regional Water Quality Control Board

Julie C. Bower, Ph.D. and Ronald S. Tjeerdema, Ph.D.

Department of Environmental Toxicology University of California, Davis

June 2016

Table of Contents

Disc	clain	ner	i
List	of F	Figures	. iv
List	of T	Tables	. iv
List	of a	cronyms and abbreviations	V
1	Intr	oduction	1
2		sic information	
3		sical-chemical data	
4		man and wildlife dietary values	
5		otoxicity data	
6		a reduction	
7		ate criterion calculation	
8		ronic criterion calculation	
9		ter quality effects	
9.		Bioavailability	
9.	2	Mixtures	
9.		Temperature, pH, and other water quality effects	
10	Coı	mparison of ecotoxicity data to derived criteria	
10).1	Sensitive species	11
10).2	Threatened and endangered species	12
10).3	Ecosystem and other studies	13
11	Har	monization with other environmental media	13
11	L.1	Bioaccumulation	13
11	L. 2	Harmonization with air and sediment criteria	14
12	Sin	nazine criteria summary	14
12	2.1	Limitations, assumptions, and uncertainties	14
12	2.2	Comparison to national standard methods	15
12	2.3	Final criteria statement	17
Ack	now	rledgements	18
Refe	eren	ces	19
Data	a Ta	bles	25
App	end	ix A – Fit Test Calculations	34
App	end	ix B – Aqueous Toxicity Data Summaries	36

Appendix B1 – Aqueous Toxicity Studies Rated RR	37
Appendix B2 – Wildlife Toxicity Studies Rated R	57
Appendix B3 – Mesocosm studies rated R	
Appendix B4 – Studies rated RL, LR, LL	64
Appendix B5 – Mesocosm studies rated L	99
Appendix B6 – Aqueous studies rated N	
List of Figures	
Figure 1 Structure of simazine	8
List of Tables	
Table 1 Bioconcentration factors (BCF) for simazine	3
Table 2 Simazine hydrolysis and photolysis and other degradation	3
Table 3 Supplemental acute data rated RL, LR, LL.	
Table 4 Final chronic plant toxicity data set for simazine.	
Table 5 Acceptable reduced chronic data rated RR	
Table 6 Supplemental chronic plant toxicity data for studies rated RL, LR, or LL	
Table 7 Supplemental chronic animal toxicity data for studies rated RL, LR, or LL	
Table 8 Acceptable multispecies field, semi-field, laboratory, microcosm, mesocos	
studies.	
Table 9 Threatened, Endangered, or Rare Species Predicted values by ICE	
Table 10 US EPA Aquatic Life Benchmarks.	33

List of acronyms and abbreviations

AF Assessment factor

APHA American Public Health Association

ASTM American Society for Testing and Materials

BAF Bioaccumulation Factor
BCF Bioconcentration Factor
BMF Biomagnification Factor
CAS Chemical Abstract Service

CDFG California Department of Fish and Game

CSIRO Commonwealth Scientific and Industrial Research Organization, Australia

CVRWQCB Central Valley Regional Water Quality Control Board

DPR Department of Pesticide Regulation

EC_x Concentration that affects x% of exposed organisms

FDA Food and Drug Administration

FT Flow-through test

 IC_x Inhibition concentration; concentration causing x% inhibition

ICE Interspecies Correlation Estimation

IUPAC International Union of Pure and Applied Chemistry

K Interaction Coefficient K_H Henry's law constant

 K_{ow} Octanol-Water partition coefficient K_p or K_d Solid-Water partition coefficient

 LC_x Concentration lethal to x% of exposed organisms

 LD_x Dose lethal to x% of exposed organisms

LL Less relevant, Less reliable study

LOEC Lowest-Observed Effect Concentration

LR Less relevant, Reliable study

MATC Maximum Acceptable Toxicant Concentration

Not relevant or Not reliable study

n/a Not applicable

NOEC No-Observed Effect Concentration

NR Not reported

OECD Organization for Economic Co-operation and Development

pKa Acid dissociation constant
RL Relevant, Less reliable study
RR Relevant and Reliable study

S Static test

SMAV Species Mean Acute Value

SR Static renewal test

SSD Species Sensitivity Distribution
TES Threatened and Endangered Species

US United States

USEPA United States Environmental Protection Agency

1 Introduction

A methodology for deriving freshwater water quality criteria for the protection of aquatic life was developed by the University of California - Davis (TenBrook et al. 2009a). The need for a methodology was identified by the California Central Valley Regional Water Quality Control Board (CVRWQCB 2006) and findings from a review of existing methodologies (TenBrook & Tjeerdema 2006, TenBrook et al. 2009b). The UC-Davis methodology is currently being used to derive aquatic life criteria for several pesticides of particular concern in the Sacramento River and San Joaquin River watersheds. The methodology report (TenBrook et al. 2009a) contains an introduction (Chapter 1); the rationale of the selection of specific methods (Chapter 2); detailed procedure for criteria derivation (Chapter 3); and a criteria report for a specific pesticide (Chapter 4). This criteria report for simazine describes, section by section, the procedures used to derive criteria according to the UC-Davis methodology. Also included are references to specific sections of the methodology procedure detailed in Chapter 3 of the report so that the reader can refer to the report for further details (TenBrook et al. 2009a).

2 Basic information

Chemical: Simazine (Fig. 1)

CAS: 6-chloro-N,N'-diethyl-1,3,5-Triazine-2,4-diamine

CAS Number: 122-34-9 USEPA PC Code: 080807 CA DPR Chem Code: 531

IUPAC: 6-chloro-N2,N4-diethyl-1,3,5-triazine-2,4-diamine

Chemical Formula: C₇H₁₂Cl₁N₅

Figure 1 Structure of simazine

(source: USEPA 2015)

Trade names: Aquazine, Caliber, Cekusan, Cekusima, Framed, Gesatop, Primatol S, Princep, Simadex, Simanex, Sim-Trol, Tanzine, and Totazine

3 Physical-chemical data

Molecular Weight

201.657 (http://webbook.nist.gov/cgi/inchi/InChI%3D1S/C7H12ClN5/c1-

3-9-6-11-5(8)12-7(13-6)10-4-2/h3-4H2%2C1-2H3%2C(H2%2C9%2C10%2C11%2C12%2C13))

Density

1.3 g/mL (PPDB 2016)

Water Solubility

5 mg/L at unknown temperature (Geigy Agricultural Chemicals 1960)

5 mg/L at unknown temperature (Gysin and Knusli 1960)

5 mg/L at 20°C (PPDB 2016) 6.2 mg/L at 20°C (USEPA 2015) 6.2 mg/L at 20°C (Tomlin 1997)

Geometric mean: 5.45 mg/L

Melting Point

112°C (USEPA 2015) 226°C (USEPA 2015) 225-227°C (Decomposes) (Tomlin 1997) Decomposes before melting (PPDB 2016)

Geometric mean: 179°C

Vapor Pressure

0.122 mPa at 25°C (USEPA 2015) 0.00295 mPa at 25°C (USEPA 2015) 0.00081 mPa at 25°C (PPDB 2016) **Geometric mean:** 0.014 mPa 25 °C

Henry's constant (K_H)

4.129 x 10 ⁻⁹ Pa m³ mol⁻¹ (USEPA 2015) 9.42 x 10 ⁻¹⁰ Pa m³ mol⁻¹ (USEPA 2015) 5.56 x 10 ⁻¹⁰ Pa m³ mol⁻¹ (PPDB 2016)

Geometric mean: 1.29 -9 Pa m³ mol⁻¹

Organic Carbon Sorption Partition Coefficients (log K_{oc})

All values from USEPA 2015

2.1661.9202.100

Geometric mean: 2.059

$Log K_{ow}$

*Values referenced from the BioByte Bio-Loom program (2015)

- 2.40 (USEPA 2015)
- 2.18 (USEPA 2015)
- 2.30 (PPDB 2016)
- 2.16 (Brown and Flagg 1981*)
- 2.26 (Finizio et al. 1991 *)
- 1.95 (Worthing and Hance 1990*)
- 2.18 (Liu and Qian 1995*)
- 2.07 (Finizio et al. 1997 *)
- 2.03 (Wu et al. 1998*)
- 2.11 (Kaune et al. 1998*)
- 1.86 (Kaune et al. 1998*)
- 2.03 (Kaune et al. 1998*)
- 1.49 (Donovan and Pescatore 2002*)

Geometric mean: 2.07

Bioconcentration Factor

Table 1 Bioconcentration factors (BCF) for simazine NR: not reported; values are on a wet weight basis and are not lipid-normalized.

Species	BCF	Exposure	Reference
NR	3.877	NR	USEPA 2015
NR	11.36	NR	USEPA 2015
NR	221	NR	PPDB 2016
NR, fish	<10	NR	USEPA 1995

Environmental Fate

Table 2 Simazine hydrolysis and photolysis and other degradation.

NR: not reported.

	Half- life (h or d)	Water	Temp (°C)	pН	Reference
Hydrolysis	>28 d	Aqueous buffer	25	5, 7, 9	Gold 1973
Aqueous Photolysis	382 d	Aqueous buffer	25	7.0	Das 1989
dark	108.8 d	*Natural pond water with underlying sediment	*25	*7.3	Burton 1993
Biodegradation (aerobic)	158 d	Loamy sand	24	NR	Spare 1993
	91 d	Sandy loam	25	NR	Cohen 1993
	90 d	Silt loam	20	NR	Müller- Kaller 1993
Biodegradation (anaerobic)	664 d	Sandy clay	25	NR	Spare 1987

4 Human and wildlife dietary values

There are no FDA action levels for simazine in food (USFDA 2000) and there are no EPA pesticide tolerances set for any aquatic species (USEPA 2007, 2012).

Wildlife LC₅₀ values (dietary) for animals with significant food sources in water

The US EPA Environmental Risk Assessment for the Reregistration of Simazine (USEPA 2006) states that simazine is practically nontoxic to birds for acute exposures. The report does not include LC_{50} toxicity values for wildlife due to a lack of definitive ecotoxicity values available at the time of publication.

No LC₅₀ data was available for wildlife species with significant food sources in water during the present report preparation. If highly rated measured data for mallard duck becomes available in the future, it should be examined to determine the potential risk to wildlife.

Wildlife dietary NOEC values for animals with significant food sources in water

The Reregistration report (USEPA 2006) does not include NOEC toxicity values for wildlife due to a lack of definitive ecotoxicity values available at the time of publication. Beavers et al. (1994) reported a NOEC value of 150 mg/kg for mallard based on female body weight and egg production. A dietary study using 80% simazine in formulation reviewed by Rieder (1965) reported a NOEC value of 1,800 mg/kg while a later Rieder study (1974) found no adverse effects, resulting in a NOEC value that exceeds the highest tested concentration of 20 mg/kg.

5 Ecotoxicity data

Approximately 23 original studies on the effects of simazine on aquatic life were identified and reviewed. In the review process, many parameters were rated for documentation and acceptability for each study, including, but not limited to: organism source and care, control description and response, chemical purity, concentrations tested, water quality conditions, and statistical methods (see Tables 3.6, 3.7, 3.8 in TenBrook et al. 2009a). Single-species effects studies that were rate as relevant (R) or less relevant (L) according to the method (Table 3.6) were summarized in data summary sheets. Information in these summaries was used to evaluate each study for reliability, using the rating systems described in the methodology (Tables 3.7 and 3.8, section 3-2.2, TenBrook et al. 2009a), to give a reliability rating of reliable (R), less reliable (L), or not reliable (N).

Studies of the effects of simazine on mallard ducks were rated for reliability using the terrestrial wildlife evaluation. Mallard studies rated as reliable (R) or less reliable (L) were used to consider bioaccumulation. Three studies for mallard duck rating R were located in the literature and they are summarized in Section 4.

Copies of completed summaries for all aquatic studies are included in the Appendix of this report. All data rated as acceptable (RR) or supplemental (RL, LR, LL) for criteria derivation are summarized in Tables 3 - 10, found at the end of this report. Acceptable studies rated as RR are used for numeric criteria derivation, while supplemental studies rated as RL, LR or LL are used for evaluation of the criteria to check that they are protective of particularly sensitive species and threatened and endangered species. These considerations are reviewed in section 12 and 14 of this report, respectively. Studies that were rated not relevant (N) or not reliable (RN or LN) were not used for criteria derivation.

One highly rated microcosm study was identified and reviewed. This study used a formulation of simazine but rated R according to the methodology and is listed in Appendix B3. It was used as supporting data in Section 13 to evaluate the derived criteria to ensure that they are protective of ecosystems. An additional microcosm study was reviewed that used a simazine formulation and was included as supplemental data in Appendix B5.

Evaluation of aquatic animal data

Using the data evaluation criteria (section 3-2.2, TenBrook et al. 2009a), there were no acute studies rated reliable and relevant for acute criterion derivation. Two acute toxicity animal values for two taxa from seven studies were rated RL, LL, or LR and were used as supplemental information for evaluation of the derived acute criteria in the Sensitive Species section 12 (Table 3). There were no chronic animal toxicity values rated RR. Four chronic toxicity animal values from four studies were rated RL, LL, or LR (Table 7).

Evaluation of aquatic plant data

Plant data were used to derive the chronic criterion instead of chronic animal data because simazine is an herbicide and plants are the most sensitive taxa (section 3-4.3, TenBrook et al. 2009a). All plant studies were considered chronic because the typical endpoints of growth or reproduction are inherently chronic. Five studies yielding five plant toxicity values were rated RR for the chronic criterion derivation (Tables 4).

Plant studies are more difficult to interpret than animal data because a variety of endpoints may be used, but the significance of each one is less clear. In this methodology, only endpoints of growth or reproduction (measured by biomass) and tests lasting at least 24-h had the potential to be rated highly and used for criteria calculation, which is in accordance with standard methods (ASTM 2007a, 2007b; USEPA 1996). The plant studies were rated for quality using the data evaluation criteria described in the methodology (section 3-2.2, TenBrook et al. 2009a).

There are several endpoints listed in the tables for plant data. The endpoints are explained here for clarity and the description includes if the endpoint is clearly linked to survival, growth, or reproduction.

Growth inhibition: All of these endpoints are relative to a control growth measurement. Depending on the plant it may have been measured by direct cell counts with a hemacytometer, cell counts with a spectrophotometer, cell counts with an electronic particle counter, chlorophyll concentration measured by absorbance, turbidity measured by absorbance, or number of fronds (*Lemna spp.*). In all cases, growth of exposed samples was compared statistically to controls.

Growth Rate: Biomass of macrophytes was measured before and after exposure to calculate a growth rate as (final mass-initial mass)/initial mass x 100. This endpoint is very similar to growth inhibition, except it is expressed as a positive effect, while growth inhibition is expressed a negative effect. In all cases, growth rate of exposed samples was compared statistically to controls.

6 Data reduction

Multiple toxicity values for simazine for the same species were reduced down to one species mean acute value (SMAV) or one species mean chronic value (SMCV) according to procedures described in the methodology (section 3-2.4, TenBrook et al. 2009a). There were no acceptable acute or chronic data reduced for simazine. The final data set for simazine includes chronic plant values shown in Tables 4.

7 Acute criterion calculation

The acute criterion is calculated with acute animal toxicity data only, because plant toxicity tests are always considered chronic (section 3-2.1.1.1, TenBrook et al. 2009a). An acute criterion could not be calculated for simazine due to a lack of highly rated studies. There were no acute animal studies that rated RR. Two acute animal studies were rated as supplemental (RL, LR, or LL), shown in Table 3. These studies reported only estimated minimum values, the lowest being $> 3,500 \,\mu\text{g/L}$ for *Daphnia magna* (Marchini 1988). This value is presented here for reference only.

8 Chronic criterion calculation

Simazine is an herbicide and the chronic data in Tables 4 and 7 demonstrate that plants are the most sensitive taxa; therefore, the procedure for derivation of the chronic criterion of an herbicide was followed (section 3-4.3, TenBrook et al. 2009a). The chronic criterion is derived to be protective of plants, but will also likely be protective of animals, which are less sensitive to simazine. Five chronic toxicity values were available for five different species of vascular plants or alga, so a distribution was fit to the available toxicity data (part 1, section 3-4.3, TenBrook et al. 2009a). The log-logistic species sensitivity distribution (SSD) procedure (section 3-3.2.2, TenBrook et al. 2009a) was used for the chronic criterion calculation because there were not more than eight acceptable chronic toxicity values available in the simazine data set (Table 4).

At least five acceptable chronic toxicity values were available and fulfilled the five taxa requirements of the SSD procedure for an herbicide (section 3-4.3 section 1, TenBrook et al. 2009a). The method requires data for at least five different species of alga or vascular aquatic plants. This data set includes three alga and two vascular plants. The five SMCVs in the acceptable data set (Table 4) were plotted in a histogram (Figure 2). The data do not appear to be bimodal, but there are small gaps between in the data in the lower end. The log-logistic SSD procedure was used to derive 5th percentile values (median and lower 95% confidence limit), as well as 1st percentile values (median and lower 95% confidence limit). The median 5th percentile value is recommended for use in criteria derivation by the methodology because it is the most robust of the distributional estimates (section 3-3.2, TenBrook et al. 2009a). Comparing the median estimate to the lower 95% confidence limit of the 5th percentile values, it can be seen that the first significant figures of the two values are different (22.81 vs. 5.38 μg/L). Because there is uncertainty in the first significant digit, the final criterion will be reported with one significant digit (section 3-3.2.6, TenBrook et al. 2009a).

The ETX 1.3 Software program (Aldenberg 1993) was used to fit a log-logistic distribution to the data set, which is plotted with the chronic values in Figure 3. This distribution provided a satisfactory fit (Appendix A: Fit test calculations) according to the fit test described in section 3-3.2.4 of TenBrook et al. (2009a). No significant lack of fit was found ($\chi^2_{2n} = 0.1988$) using the fit test based on cross validation and Fisher's combined test (section 3-3.2.4, TenBrook et al. 2009a), indicating that the data set is valid for criteria derivation.

Log-logistic distribution

HC5 Fitting Parameter Estimates: $\alpha = 1.8008$, β (median) = 0.1504, β (lower 95% CI) = 0.3633.

5th percentile, 50% confidence limit: 22.81 µg/L

5th percentile, 95% confidence limit: 5.384 µg/L

1st percentile, 50% confidence limit: 12.90 μg/L

1st percentile, 95% confidence limit: 1.353 µg/L

Recommended chronic value = $22.81 \mu g/L$ (median 5th percentile value)

Chronic criterion = $20 \mu g/L$

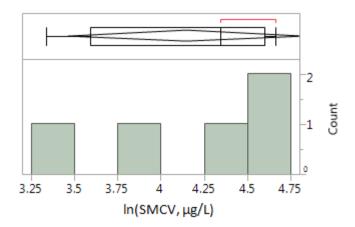


Figure 2 Histogram of acceptable simazine chronic data.

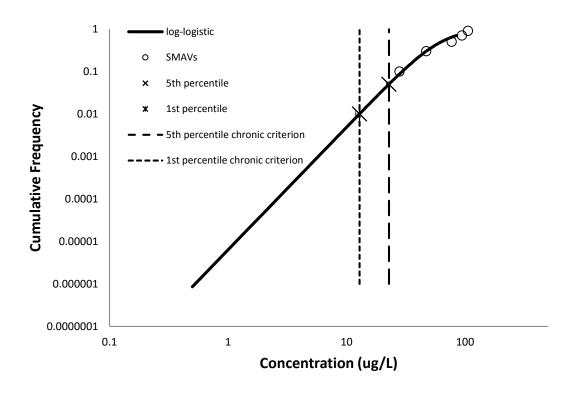


Figure 3 Fit of the log-logistic species sensitivity distribution to the chronic data set.

The median 5th percentile chronic value with the lower 95% confidence limit and the median 1st percentile chronic value with the lower 95% confidence limit are each displayed. The chronic water quality criteria calculated with the median 5th percentile and median 1st percentile values are displayed as vertical lines.

Two of the chronic toxicity values were from closely related plants, *Lemna gibba* and *Lemna minor*. The methodology states that only the species must differ for the SSD

procedure (part 1, section 3-4.3, TenBrook et al. 2009a). Because only the minimum number of chronic toxicity values were available for the SSD procedure, the lowest NOEC value was determined for comparison. The methodology instructs that in the absence of acceptable data to fit a distribution, the chronic criterion is equal to the lowest NOEC from an important alga or vascular aquatic plant species that has measured concentrations and a biologically relevant endpoint (part 2, section 3-4.3, TenBrook et al. 2009a). Acceptable toxicity data for the aquatic plant *Anabena flos-aquae* (Swigert 1992) is shown in Table 6 with a NOEC of 20 μ g/L. The chronic criterion calculated by the log-logistic SSD procedure is equal to this NOEC. Therefore *A. flos-aquae* will likely be protected and the chronic criterion will not be adjusted downward.

Chronic criterion = $20 \mu g/L$

9 Water quality effects

9.1 Bioavailability

No studies were found regarding the aquatic bioavailability of simazine.

9.2 Mixtures

Simazine can occur in the environment with other herbicides of similar or different modes of action. Simazine is an s-triazine pesticide that acts as a photosystem II (PSII) inhibitor. Other widely used herbicides, such as the phenylurea class, are also PSII inhibitors, but have different binding sites than the triazine herbicides. The concentration addition model and the non-additive interaction model are the only predictive mixture models recommended by the methodology (section 3-5.2, TenBrook et al. 2009a), so other models found in the literature will not be considered for compliance.

Several studies have confirmed that toxicity of a mixture of herbicides that are PSII-inhibitors can be predicted by the concentration addition method (Faust et al. 2000) and 2001, Drost et al. 2003, Wilkinson et al. 2015). Faust et al. (2000) studied a mixture of 18 triazines with identical mechanisms of action with a unicellular green alga Scenedesmus vacuolatus and found that the combined toxicity could be predicted by concentration addition. Faust et al. (2001) again used a mixture of 18 different s-traizine herbicides with unicellular green alga Scenedesmus vacuolatus to show that the toxic effects of the mixture exceeded that of the most active ingredient alone. Even nonsignificant effect concentrations of the herbicides contributed to mixture toxicity. Concentration addition predictions were accurate for all effect levels and concentration ratios of herbicides. Drost et al. (2003) reported that concentration addition prediction was valid for a mixture of four s-triazines with Lemna minor. Near complete recovery of growth occurred within three days when the plants were moved to pesticide-free growth medium. Concentration addition was also valid in seawater as reported by Wilkinson et al. (2015). In this study, a mixture of ten photosystem II herbicides of similar mechanism of action was tested on the seagrass *Halophila ovali*. It was shown that other approaches

should be used for systems containing mixtures of herbicides with dissimilar mechanisms of action.

Faust et al. (1993) studied binary mixtures of simazine and a variety of herbicides of similar and dissimilar modes of action on the unicellular green alga *Chlorella fusca*. In all but one case the mixtures exhibited toxicity consistent with the concentration addition model. When combined with methabenzthiazuron, a benzoylthiazolylurea photosystem II inhibitor, the effect was greater than predicted by the concentration addition model by 30%.

Kumar and Han (2011) tested mixture toxicity of four herbicides with similar modes of action on the F_v/F_m chlorophyll fluorescence of the PSII pathway of *Lemna* sp following a 96-hour exposure. A factorial design of high and low concentrations of binary mixtures was used. Simazine mixtures resulted in a range of toxicological effects, depending on the herbicide and the relative strength of the mixture. Additive effects were observed with low levels of both simazine and diuron whereas antagonism was observed for low levels of simazine mixed with both high and low levels of atrazine. Synergistic effects resulted from most combinations of simazine and hexazinone except for high concentrations of simazine and hexazinone where antagonism was observed. Coefficients of interaction were not calculated in this study so adjustments to the criterion for compliance cannot be considered.

Lydy and Austin (2004) assessed the toxicity to the midge *Chironomus tentans* of nine pesticides with various modes of action, including organophosphate insecticides, triazines herbicides, organochlorine insecticides, substituted urea herbicides, and triazinone herbicides. While simazine was not found to be toxic to the midge, when in combination with organophosphate insecticides azinphos methyl, chlorpyrifos, diazinon, and methidathion a greater-than-additive response was observed. Simazine synergistically increased chlorpyrifos and methidathion toxicity by 1.8 and 2.4 times, respectively.

Binary mixtures of five herbicides of identical modes of action and two breakdown products were tested with three algae, *Navicula* sp., *Cylindrotheca closterium*, and *Nephroselmis pyriformis*, and the tropical diatom, *Phaeodactylum tricornutum* in a study by Magnusson et al. (2010). All mixtures were predicted by concentration addition toxicity.

Perez et al. (2011) exposed the alga *R. subcapitata* to binary mixtures of three striazines and one chloroacetabilide herbicide. These chemical classes have different modes of action. Similar acting binary mixtures were predicted by concentration addition except for a synergism due to atrazine when it was dominant. Antagonism due to dominant simazine was observed in the simazine/metolachlor mixture when the independent action model was applied.

Villa et al. 2012 assessed the toxicity of 84 chemicals of various modes of action on the bacterium *Vibrio fischeri*. The chemicals included narcotics, herbicides,

insecticides, and fungicides and were tested in eight complex mixtures although herbicides were not combined with other classes. The chemical classes of the herbicides included phenylureas, triazines, pyridazinones, triazinones, and anilides. The concentration addition model was found to be valid for the herbicide mixture.

Schuler et al. (2005) studied the effect of simazine on the organophosphate insecticides on the midge *Chironomus tentans*. Simazine potentiated insecticide toxicity in a dose-dependent manner. Synergistic ratios ranged from 1.0-2.5 in binary mixtures with diazinon and 1.1-1.8 with chlorpyrifos. In a similar study, Trimble and Lydy (2006) studied the effect of simazine on chlorpyrifos on the amphipod crustacean *Hyalella azteca*. Simazine caused a significant effect on chlorpyrifos toxicity in binary mixture tests, resulting in a synergistic ratio of 1.18. Torres and O'Flaherty (1976) tested combinations of three herbicides with an organophosphate insecticide on six species of algae. The effects ranged from stimulatory to inhibitory and no clearly defined results were presented regarding mixture toxicity models.

In summary, when simazine is detected with other s-triazine PSII-inhibitor herbicides the toxicity should be predicted by the concentration addition model. In some cases this model will not be valid and synergism or antagonism will be observed. There are no multi-species coefficients of interaction reported in the literature, so the non-additive interaction model cannot be used to assess water quality criteria compliance when other types of contaminants are present. No studies on aquatic organisms were identified in the literature that could provide a quantitative means to consider mixtures of simazine with other classes of pesticides.

9.3 Temperature, pH, and other water quality effects

There were no studies available that examined the effects of temperature or pH on simazine toxicity in the aqueous environment. As simazine is a very weak base, pH is not expected to have a significant effect on the chemical structure in the range of conditions found in natural freshwater environments.

10 Comparison of ecotoxicity data to derived criteria

10.1 Sensitive species

The derived criteria were compared to toxicity values for the most sensitive species in both the acceptable (RR) and supplemental (RL, LR, LL) data sets to ensure that these species will be adequately protected (section 3-6.1, TenBrook et al. 2009a).

There were no highly rated (RR) acute studies available and only two studies are included as supplemental data (RL, LR, or LL). The lowest acute value in the data sets rated RL, LR, or LL (Table 3) is $>3,500~\mu g/L$ for *Daphnia magna* (Marchini 1988). The value was reported as a minimum and confidence intervals were not calculable. This study rated LL because the control response, concentrations, and water parameters were

not reported, and the toxicity value is censored. Therefore this study is less reliable for the purposes of the methodology, but it is still a relevant toxicity study. This study tested an aquatic species that resides in North America with the endpoint and exposure duration fit into the acute test definition in the methodology (section 3-2.1.1.1). The next lowest acute value is $>4,300~\mu g/L$ for the saltwater fish *Cyprinodon variegatus* which is rated LR (Murphy 1992). It was reported as a minimum value. These values are references only because no acute criterion could be calculated for simazine at this time.

The derived chronic criterion (20 μ g/L) is lower than the MATC values of all chronic data that was highly rated (Table 4). The lowest NOEC in the data sets is 20 μ g/L for growth rate of the cyanobacterium *Anabena flos-aquae* (Swigert 1992). The chronic criterion should be adequately protective of this species. Simazine is an herbicide and it is shown that plants will be more sensitive than animals, therefore the chronic criterion should be adequately protective of both plant and animal species.

10.2 Threatened and endangered species

The derived criteria are compared to measured toxicity values for threatened and endangered species (TES), as well as to predicted toxicity values for TES, to ensure that they will be protective of these species (section 3-6.3, TenBrook et al. 2009a). Current lists of state and federally listed threatened and endangered plant and animal species in California were obtained from the California Department of Fish and Game website (CDFG 2015). One listed animal species is represented in the dataset. Five Evolutionarily Significant Units of *Oncorhynchus mykiss* are listed as federally threatened or endangered throughout California. There were no toxicity values for this genus in the acute or the chronic data sets. The most comparable animals in the data set are the fish Danio rerio and Cyprinus carpio in the supplemental chronic data set, with NOEC values of 6.0 µg/L and 0.06 µg/L, respectively. It should be noted that these values were derived from histopathological endpoints rather than parameters related to growth, survival, or reproduction (section 3-2.1.1.1, TenBrook et al. 2009a). The acute data set contained an LC₅₀ of >4,300 µg/L for *C. variegatus*. These data indicate that the chronic criterion of 20 µg/L would not be protective of these fish species. The chronic criteria cannot be adjusted downward until additional studies are available that use appropriate endpoints and species in the *Oncorhynchus* genus or other genus listed as threatened or endangered on the state or federal level.

The USEPA interspecies correlation estimation (ICE v. 3.1; USEPA 2010) software was consulted to estimate toxicity values for the listed animals or plants represented in the acute data set by members of the same family or genus. There were no threatened or endangered species included in the acute data set. For the purposes of illustration, the correlation was performed for the only species available in the ICE, *Dapnia magna*. Table 9 summarizes the results of the ICE analyses.

No plant studies used in the criteria derivation were of state or federal endangered, threatened or rare species. Plants are particularly sensitive to simazine because it is an herbicide, but there are no aquatic plants listed as state or federal endangered, threatened or rare species so they could not be considered in this section.

Based on the available data and estimated values for animals, there is no evidence that the chronic criterion will be underprotective of threatened and endangered species.

10.3 Ecosystem and other studies

The derived criteria are compared to acceptable laboratory, field, or semi-field multispecies studies (rated R or L) to determine if the criteria will be protective of ecosystems (section 3-6.2, TenBrook et al. 2009a). Two mesocosm, microcosm or ecosystem (field and laboratory) studies were identified. Vervliet-Scheebaum et al. (2010) studied a variety of rooted macrophytes and natural alga in concrete ponds with tap water. The study rated R and tested a 50% simazine formulation. A NOEC of 50 μg/L based on nominal concentrations was established for growth parameters of the macrophytes *Persicaria amphibian*, *Glyceria maxima*, and *Elodea canadensis*. A NOEC of 500 μg/L was calculated for *Myriophyllum spicatum*. An *in situ* pond microcosm study by Jenkins (1990) tested a range of winter bacteria, phytoplankton, and zooplankton and rated L. It utilized a formulation containing 41.9% simazine. The natural waters used in the study included a range of species there were not well characterized. Observed effects were complex and varied by simazine concentration and species. Toxicity values were not reported.

The derived chronic criterion is a factor of four times lower than the lowest concentration tested in the Jenkins (1990) microcosm study and 3.5 times lower than the lowest measured concentration in the Vervliet-Scheebaum et al. (2010) study. It is therefore expected that the chronic criterion is adequately protective of the tested species.

11 Harmonization with other environmental media

11.1 Bioaccumulation

Bioaccumulation was assessed to ensure that the derived criteria will not lead to unacceptable levels of simazine in food items (section 3-7.1, TenBrook et al. 2009a). Simazine has a log K_{ow} of 2.07 (Section 3), a K_d of 0.03-4.28 depending on material (Hodges and Talbert 1990, Sannino et al. 1999, Beltran et al. 1998, Bereton et al. 1999, Reddy et al. 1992, Barriuso et al. 1997, Cox et al. 2000), and a molecular weight of 201.66, which may indicate some degree of bioaccumulative potential. There are no FDA action levels for simazine in food (USFDA 2000), and there are no EPA pesticide tolerances set for any aquatic species (USEPA 2007, 2012). Bioconcentration of simazine has been measured in unknown species (Table 1).

To check that these criteria are protective of terrestrial wildlife that may consume aquatic organisms, a bioaccumulation factor (BAF) was used to estimate the water concentration that would roughly equate to a reported toxicity value for such terrestrial wildlife ($LC_{50, \, oral \, predator}$). These calculations are further described in section 3-7.1 of the methodology (TenBrook et al. 2009a). The BAF of a given chemical is the product of the

BCF and a biomagnification factor (BMF), such that BAF=BCF*BMF. No BMF value was found for simazine. Chronic dietary toxicity values are preferred for this calculation. The BAF and BCF values available were either from an estimation modeling program (USEPA 2015) or the value origin was not reported (PPDB 2015). The lowest dietary value for mallard was 150 mg/kg (Beavers 1994). A BCF of 21.4 L/kg (USEPA 2015, PPDB 2015) were used as an example estimation of bioaccumulation in the environment. No BMF value was available in the literature so it was estimated two ways according to the methodology (a value of 1 both when as approximated from log K_{ow} and as approximated from BCF as in section 3-7.1 and Table 3.15 in TenBrook et al. 2009a).

$$NOEC_{water} = \frac{NOEC_{oral-predator}}{BCF_{food_item} \cdot BMF_{food_item}}$$

Mallard:
$$NOEC_{water} = \frac{150^{mg}/kg}{21.4 \frac{1}{kg} * 1} = 7.009^{mg}/L = 7,009^{\mu g}/L$$

In this example, the calculated chronic criterion (20 μ g/L) is more than three orders of magnitude below the estimated NOEC_{water} value for wildlife and is not expected to cause adverse effects due to bioaccumulation.

11.2 Harmonization with air and sediment criteria

This section addresses how the maximum allowable concentration of simazine might impact life in other environmental compartments through partitioning (section 3-7.2, TenBrook et al. 2009a). There were no sediment studies using technical products available in the literature. The Reregistration Eligibility Decision for simazine (USEPA 2006) includes only a single benthic citation that utilized an herbicide formulation. The other available sediment criterion for simazine is estimated based on partitioning from water using empirical K_{oc} values. These range from 1.920 µg/L to 2.166 µg/L (USEPA 2015). There are no other federal or state sediment or air quality standards for simazine (CARB 2008; CDWR 1995), nor is simazine mentioned in the NOAA sediment quality guidelines (NOAA 1999). For biota, the limited data on bioconcentration or biomagnification of simazine is addressed in section 15.

12 Simazine criteria summary

12.1 Limitations, assumptions, and uncertainties

The assumptions, limitations and uncertainties involved in criteria generation are available to inform environmental managers of the accuracy and confidence in criteria (section 3-8.0, TenBrook et al. 2009a). Chapter 2 of the methodology (TenBrook et al. 2009a) discusses these points for each section as different procedures were chosen, such

as the list of assumptions associated with using an SSD (section 2-3.1.5.1), and reviews them in section 2-7.0. This section summarizes any data limitations that affected the procedure used to determine the final simazine criteria.

Overall, there was a lack a highly rated aquatic plant and animal toxicity data for simazine. There was a complete lack of RR rated studies available for both acute and chronic tests for animals. The chronic plant data set contained the minimum number of values necessary for a log-logistic SSD calculation.

The most important limitation is the lack of acceptable animal data. Although simazine is an herbicide, it comes into contact with aquatic animals when applied to ponds to control aquatic weeds. The chronic data set only contained five plant values, the minimum required for a SSD calculation. The methodology requires that MATC values are used to derive chronic criterion by the SSD procedure, unless studies are available with EC_x values that show what level of x is appropriate to represent a no-effect level (section 3-2.1.1.2, TenBrook et al. 2009a). However, chronic animal data is not used for chronic criterion derivation of an herbicide, or when plants are the most sensitive taxa to a particular pesticide (3-4.3, TenBrook et al. 2009a). Although simazine is an herbicide, some animals do show sensitivity to it as seen in the supplemental chronic animal studies listed in Table 7.

Other limitations include the lack of sediment studies to assess partitioning of simazine from environmental niches other than the water column. There were no sediment studies available for simazine that utilized a technical or high purity product.

12.2 Comparison to national standard methods

This section is provided as a comparison between the UC-Davis methodology for criteria calculation (TenBrook et al. 2009a) and the current USEPA (1985) national standard. The following example simazine criteria were generated using the USEPA (1985) methodology with the data set generated in this simazine criteria report.

The USEPA acute methods have three additional taxa requirements beyond the five required by the SSD procedure of the UC-Davis methodology (section 3-3.1, TenBrook et al. 2009a). They are:

- 1. A third family in the phylum Chordata (e.g., fish, amphibian);
- 2. A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca);
- 3. A family in any order of insect or any phylum not already represented.

None of the three additional requirements could be met because there were no highly rated acute values available. Because of this lack of data, no acute criterion could be calculated according to the USEPA (1985) methodology.

According to the USEPA (1985) methodology, the chronic criterion is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value.

To calculate the Final Chronic Value, animal data is used and the same taxa requirements must be met as in the calculation of the acute criterion (section III B USEPA 1985). There are no chronic animal data available that rated RR, thus the final chronic value could not be determined. The missing taxa are as follows:

- 1. the family Salmonidae in the class Osteichthyes
- 2. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
- 3. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
- 4. a planktonic crustacean (e.g., cladoceran, copepod, etc.)
- 5. a benthic crustacean (e.g., oatracod, isopod, aatphipod, crayf isb, etc.)
- 6. an insect (a.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
- 7. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
- 8. a family in any order of insect or any phylum not already represented.

The Final Plant Value is calculated as the lowest result from a 96-hr test conducted with an important plant species in which the concentrations of test material were measured and the endpoint was biologically important. Only one of the plant toxicity values in the RR data set (Table 4) is for a 96-hr test; the others are longer ranging from five to 14 days. The lowest NOEC reported is $20~\mu g/L$ for *Anabena flosaquae* (Swigert 1992). This test has an exposure duration that is one day longer than the specified duration.

Final Plant Value = lowest result from a plant test = $20 \mu g/L$

The Final Residue Value is calculated by dividing the maximum permissible tissue concentration by an appropriate bioconcentration or bioaccumulation factor. A maximum allowable tissue concentration is either (a) a FDA action level for fish oil or for the edible portion of fish or shellfish, or (b) a maximum acceptable dietary intake based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or long-term wildlife field study. There are no FDA action levels for simazine in food (USFDA 2000) and there are no EPA pesticide tolerances set for any aquatic species (USEPA 2007, 2012). The lowest dietary NOEC of 150 mg/kg (Beavers et al. 1994) was the lowest wildlife dietary toxicity value available. A BCF of 21.4 for unknown species (Table 1) is used to calculate the Final Residue Value.

```
Final Residue Value = maximum acceptable dietary intake \div BCF
= 150 mg/kg \div 21.4 L/kg
= 7.009 mg/L
= 7,009 \mug/L
```

The Final Plant Value is lower than the Final Residue Value. A Final Chronic Value cannot be calculated. Therefore the chronic criterion by the USEPA (1985) methodology for simazine would be 20 μ g/L. The example chronic criterion is equal to the chronic criterion derived by the UC Davis methodology.

12.3 Final criteria statement

The final criteria statement is:

Aquatic life in the Sacramento River and San Joaquin River basins should not be affected unacceptably if the four-day average concentration of simazine does not exceed $20 \mu g/L$ more than once every three years on the average.

A limit for the one-hour average concentration to occur not more than once every three years on the average could not be determined. Although the criteria were derived to be protective of aquatic life in the Sacramento and San Joaquin Rivers, these criteria would be appropriate for any freshwater ecosystem in North America, unless species more sensitive than are represented by the species examined in the development of these criteria are likely to occur in those ecosystems.

An acute criterion could not be calculated due to a lack of highly rated studies. Discussion of the acute criterion are included in section 7. Supplemental acute data are shown in Table 3.

Details of the chronic criterion calculation are described in section 8 and chronic plant data are shown in Table 6. The chronic criterion was derived to only be protective of plants, but will also likely be protective of animals, which are less sensitive to simazine. A log-logistic SSD was fit to the highly rated plant values to derive the criterion. The chronic criterion was calculated with the minimum amount of data required for a SSD. Plant toxicity data is essential when considering simazine usage and regulations because plants and algae are the most sensitive taxa, however, plant data can be difficult to interpret. The chronic criterion was derived using the best data available, and firm evidence that could support lowering criteria was not found. The criteria should be updated whenever new relevant and reliable data is available.

There are no established water quality criteria for simazine with which to compare the criteria derived in this report. The US EPA has several aquatic life benchmarks established for simazine, shown in Table 10, to which the derived criteria in this report can be compared with caution (USEPA 2014). According to the USEPA (2014), aquatic life benchmarks are not calculated following the same methodology used to calculate water quality criteria. Water quality criteria can be used to set water quality

standards under the Clean Water Act, but aquatic life benchmarks may not be used for this purpose (USEPA 2014).

The referenced acute value in this report (>3500 μ g/L) is above both the acute fish benchmark and the acute invertebrate benchmark (by factors of 1.1 and 7 times, respectively). There is no chronic fish benchmark so a comparison to the chronic criterion is not possible. The chronic criterion is ten times above the chronic benchmark for invertebrates. However, it is 6.1 times lower than the acute nonvascular plant benchmark. Because the chronic criterion was derived using only plant data, it is most comparable to the acute nonvascular plant benchmark.

Acknowledgements

This project was funded through a contract with the Central Valley Regional Water Quality Control Board of California. Mention of specific products, policies, or procedures do not represent endorsement by the Regional Board.

References

- Beavers JB, Foster JW, Mitchell LR, Jaber M. (1994) A reproduction study with the mallard. Wildlife International, Ltd., Easton, Maryland. Wildlife International, Ltd. project number 108-356. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. CA DPR 139747. EPA MRID 43576901.
- Beliles RP, Scott W, Knott W. (1965) Simazine: Acute Toxicity in Sunfish. Woodard Research Corporation, Herndon, Virginia. Submitted to Geigy Chemical Corporation, New York, N.Y. EPA MRID 25438.
- BioByte. (2015) Bio-Loom program. URL http://www.biobyte.com/bb/prod/bioloom.html
- Bionomics. (1971) Acute toxicity of some Ciba-Geigy experimental chemicals to fathead minnow (Pimephales promeles). Bionomics, Inc., Wareham Massachusetts. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 31150.
- Brown, D.S. and Flagg, E.W., 1981. Empirical prediction of organic pollutant sorption in natural sediments. *Journal of environmental quality*, 10(3), 382-386.
- Burton SD. (1993) Aerobic aquatic metabolism of ¹⁴C-simazine. Stillmeadow, Inc., Sugar Land, Texas. Laboratory study number 9061-92. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. USEPA MRID 43004502.
- CARB (2008) California Ambient Air Quality Standards (CAAQS). California Air Resources Board, Sacramento, CA.
- CDFG (2015) State and federally listed threatened and endangered plant and animal species in California. URL http://www.dfg.ca.gov/wildlife/nongame/t_e_spp/
- CDWR (1995) Compilation of Sediment and Soil Standards, Criteria, and Guidelines.

 California Department of Water Resources, State of California, The Resources Agency, Sacramento, CA. URL

 1995.pdf
- Cohen SP. (1993) Aerobic soil metabolism of simazine. Pittsburgh Environmental Research Laboratory, Inc. Laboratory study number ME 9100139. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. USEPA MRID 43004501.
- Das YT. (1989) Photodegradation of [triazine (U)-¹⁴C] simazine in aqueous solution buffered at pH 7 under artificial sunlight. Innovative Scientific Services, Inc., Piscataway, New Jersey. Laboratory project number 89040. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. USEPA MRID 42503708.
- Donovan S, Pescatore M J. (2002) Method for measuring the logarithm of the octanol—water partition coefficient by using short octadecyl—poly(vinyl alcohol) high-performance liquid chromatography columns. *Journal of Chromatography A*, 952, 47-61
- Drost W, Backhaus T, Vassilakaki M, Grimme LH. (2003) Mixture toxicity of s-triazines to Lemna minor under conditions of simultaneous and sequential exposure. *Fresenius Environmental Bulletin*, 12 (6), 601-607.

- Fairchild JF, Ruessler DS, Haverland PS and Carlson AR. (1997) Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides. Archives of Environmental Contamination and Toxicology, 32(4), 353-357.
- Faust M, Altenburger R, Backhaus T, Boedeker W, and Grimme LH. (1993) Additive effects of herbicide combinations on aquatic non-target organisms. *The Science of the Total Environment, Supplement*, 941-952.
- Faust M, Altenburger R, Backhaus T, Bödeker W, Scholze M, and Grimme LH. (2000) Predictive assessment of the aquatic toxicity of multiple chemical mixtures. *Journal of environmental quality*, 29(4), 1063-1068.
- Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Hamer V, Scholze M, Vighi M, Grimme LH. (2001). Predicting the joint algal toxicity of multi-component s-triazine mixtures at low-effect concentrations of individual toxicants. *Aquatic Toxicology*, 56(1), 13-32.
- Finizio A, DiGuardo A, Arnoldi A, Vighi M, Fanelli R. (1991) Different approaches for the evaluation of K_{OW} for *s*-triazine herbicides. *Chemosphere*, 23, 801-812.
- Foster S, Thomas M and Korth W. (1998) Laboratory-derived acute toxicity of selected pesticides to Ceriodaphnia dubia. Australasian Journal of Ecotoxicology, 4(1), 53-59
- Geigy Agricultural Chemicals. (1960) Simazine herbicides for agricultural use. Herbicide Technical Bulletin 60-1 (revised). Ardsley, New York.
- Goldsborough LG and Robinson GGC. (1983) The effect of two triazine herbicides on the productivity of freshwater marsh periphyton. *Aquatic Toxicology*, 4(2), 95-112.
- Gold B, Baly K, Hofberg A. (1973) Hydrolysis of simazine in aqueous solution. Ciba-Geigy Corporation, Greensboro, North Carolina. USEPA MRID 27856.
- Gysin H and Knusli E. (1960) Chemistry and herbicidal properties of triazine derivatives. In Metcalf, R. L. (ed.)
- Gurney SE and Robinson GGC. 1989. The influence of two tiazine herbicides on the productivity, biomass and community composition of freshwater marsh periphyton. *Aquatic Botany*, 36: 1-22.
- Hernando MD, De Vettori S, Bueno, MM and Fernández-Alba AR. (2007) Toxicity evaluation with Vibrio fischeri test of organic chemicals used in aquaculture. *Chemosphere*, 68(4), 724-730.
- Jenkins DG and Buikema AL. (1990) Response of a winter plankton food web to simazine. *Environmental toxicology and chemistry*, 9(6), 693-705.
- Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.
- Kaune, A., Brüggemann, R. and Kettrup, A., 1998. High-performance liquid chromatographic measurement of the 1-octanol–water partition coefficient of striazine herbicides and some of their degradation products. Journal of chromatography A, 805(1), 119-126.
- Kuc WJ. (1976) Acute toxicity of simazine technical, batch # FL-750336, to the rainbow trout, Salmo gairdneri. Aquatic Environmental Sciences, Tarrytown, New York. USEPA MRID 43666.

- Kumar KS and Han T. (2011). Toxicity of single and combined herbicides on PSII maximum efficiency of an aquatic higher plant, *Lemna* sp. *Toxicology and Environmental Health Sciences*, 3(2), 97-105.
- Lehman, C. (2010) Simazine-life-cycle toxicity with the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions. ABC Laboratories, Columbia, Missouri. Laboratory report number 65071. Submitted to Syngenta Crop Protection, Inc., Greensboro, North Carolina. EPA MRID 47984801.
- Liu J, Qian C. (1995) Hydrophobic coefficients of s-triazine and phenylurea herbicides. *Chemosphere*, 31, 3951-3959.
- Lydy MJ and Austin KR. (2004) Toxicity assessment of pesticide mixtures typical of the Sacramento–San Joaquin Delta using *Chironomus tentans*. Archives of Environmental Contamination and Toxicology, 48(1), 49-55.
- Ma J, Liang W, Xu L, Wang S, Wei Y, Lu J. (2001) Acute Toxicity of 33 Herbicides to the Green Alga Chlorella pyrenoidosa. Bull. Environ. Contam. Toxicol. 66,536–541
- Ma J, Xu L, Wang S, Zheng R, Jin S, Huang S & Huang Y. (2002). Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. Ecotoxicology and environmental safety, 51(2), 128-132.
- Ma J. (2002) Differential sensitivity to 30 herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. *Bulletin of environmental contamination and toxicology*, 68(2), 275-281.
- Ma J, Lin F, Wang S and Xu L. (2003) Toxicity of 21 herbicides to the green alga *Scenedesmus quadricauda*. Bulletin of environmental contamination and toxicology, 71(3), 594-601.
- Ma J, Wang S, Ma L, Chen X, Xu R. (2006) Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*. *Ecotoxicology and Environmental Safety*. 63, 456-462.
- Ma J, Tong S, Wang P and Chen J. (2010) Toxicity of Seven Herbicides to the Three Cyanobacteria *Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa*. *International Journal of Environmental Research*, 4(2), 347-352.
- Magnusson M, Heimann K, Quayle P and Negri AP. (2010) Additive toxicity of herbicide mixtures and comparative sensitivity of tropical benthic microalgae. *Marine Pollution Bulletin*, 60(11), 1978-1987.
- Marchini S, Passerini L, Cesareo D and Tosato ML. (1988) Herbicidal triazines: Acute toxicity on Daphnia, fish, and plants and analysis of its relationships with structural factors. *Ecotoxicology and environmental safety*, 16(2), 148-157.
- Müller-Kaller HM. (1993) Degradation of 14C-simazine (G 27692) in one soil incubated under various experimental conditions. RCC Umwltchemie Ag, Itingen, Switzerland. Laboratory project number 300881. Submitted to Syngenta Crop Protection, Inc., Greensboro, North Carolina. USEPA MRID 46561301.
- Murphy, D and Swigert JP. (1992) Simazine: a 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*). Wildlife International Limited, Easton, Maryland. Laboratory project number 108A-143. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503702. CA DPR 138083.

- NOAA. (1999) Sediment Quality Guidelines Developed for the National Status and Trends Program. National Oceanographic and Atmospheric Agency Office of Response and Restoration, Department of Commerce. URLhttp://archive.orr.noaa.gov/book_shelf/121_sedi_qual_guide.pdf
- Pérez J, Domingues I, Soares AM and Loureiro S. (2011) Growth rate of *Pseudokirchneriella subcapitata* exposed to herbicides found in surface waters in the Alqueva reservoir (Portugal): a bottom-up approach using binary mixtures. *Ecotoxicology*, 20(6), 1167-1175.
- Plhalova L, Haluzova I, Macova S, Dolezelova P, Praskova E, Marsalek P, Skoric M, Svobodova Z, Pistekova V and Bedanova I. (2010) Effects of subchronic exposure to simazine on zebrafish (Danio rerio). Neuroendocrinology letters, 32, 89-94.
- PPDB, The Pesticide Properties DataBase (2016), Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006-2016. URL http://sitem.herts.ac.uk/aeru/iupac/Reports/542.htm
- Rieder D (reviewer). (1965) Simazine, subacute toxicity in mallard ducks. Truslo Farm Inc., Easton, Maryland. Later became Wildlife International (laboratory). USEPA MRID 43672.
- Rieder D (reviewer). (1974) One-generation reproduction study-mallard ducks. Truslo Farm Inc., Easton, Maryland. Later became Wildlife International (laboratory). Project number 108-101. Submitted to -Geigy Corporation, Greensboro, NC. USEPA MRID 43678.
- Schuler LJ, Trimble AJ, Belden JB and Lydy MJ. (2005) Joint toxicity of triazine herbicides and organophosphate insecticides to the midge *Chironomus tentans*. *Archives of environmental contamination and toxicology*, 49(2), 173-177.
- Sleight BH. (1971) Acute toxicity of some Ciba-Geigy experimental chemicals to fathead minnows (Pimephales promelas). Bionomics, Inc., Wareham, Massachusetts. USEPA MRID 33309.
- Sleight BH. 1973. Acute toxicity of simazine to pink shrimp (Penaeus duorarum) and mud crab (Neopanope). Bionomics, Inc., Wareham, Massachusetts. Submitted to Ciba-Geigy Corporation, Ardsley, New York. EPA MRID 23331.
- Spare WC. (1987) Anaerobic aquatic metabolism of simazine. Agrisearch Incorporated, Frederick, Maryland. Laboratory project number 1230. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. USEPA MRID 40614411.
- Spare WC. (1993) Photodegradation of ¹⁴C-simazine on soil under artificial sunlight.

 Agrisearch Incorporated, Frederick, Maryland. Laboratory project number 12206.

 Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. USEPA MRID 42739101.
- Swabey YH and Schnk CF. (1963) Report on algicides and aquatic herbicides. Ontario Water Resources Commission. EPA MRID 34214.
- Swigert JP. (1992a) A 5-day toxicity test with the freshwater alga (*Anabena flos-aquae*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-139. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42662401.
- Thompson SG. (1992a) Simazine a 14-day toxicity test with duckweed (*Lemna Gibba G3*). Wildlife International, Easton, Maryland. Laboratory study number 108A-

- 137. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 4253704. CA DPR 138090.
- Thompson SG. (1992b) A 5-day toxicity test with the freshwater alga (*Navicula pelliculosa*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-138. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503707. CA DPR 138087.
- Thompson, SG. (1992c) A five-day toxicity test with the marine diatom (*Skeletonema costatum*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-140. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503705. CA DPR 138088.
- Thompson, SG and Swigert, JP. (1992) A 5-day toxicity test with the freshwater alga (*Selenastrum capricornum*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-141. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503706. CA DPR 138086.
- Tomlin C (1997) *The Pesticide Manual. (A World Compendium.) 10th Edition.* The British Crop Protection Council and The Royal Society of Chemistry, Surrey, England and Cambridge, England.
- Torres AMR and O'Flaherty LM. (1976) Influence of pesticides on *Chlorella*, *Chlorococcum*, *Stigeoclonium* (Chlorophyceae), *Tribonema*, *Vaucheria* (Xanthophyceae) and *Oscillatoria* (Cyanophyceae). *Phycologia*, 15(1), 25-36.
- Trimble AJ, Lydy MJ. (2006) Effects of triazine herbicides on organophosphate insecticide toxicity Hyalella azteca. *Archives of Environmental Contamination and Toxicology*, 51, 29-34.
- USEPA (1985) Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses, PB-85-227049. United States 37 Environmental Protection Agency, National Technical Information Service, Springfield, VA. URL http://www.waterboards.ca.gov/waterrights/water_issues/programs/bay_delta/deltaflow/docs/exhibits/sac_rcsd/srcsd_exh1w.pdf
- USEPA (1995) National Primary Drinking Water Regulations. Contaminant Specific Fact Sheets Synthetic Organic Chemicals-Technical Version (Simazine). Docket # EPA 811-F-95-003-T. United States Environmental Protection Agency, Washington, DC, USA. URL
 - <a href="http://nepis.epa.gov/Exe/ZyNET.exe/20001S1W.TXT?ZyActionD=ZyDocument&Client=EPA&Index=1995+Thru+1999&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C95thru99%5CTxt%5C00000001%5C20001S1W.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-
 - &MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=p%7Cf&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL#>
- USEPA (2006) Reregistration Eligibility Decision (RED), Simazine. EPA738-R-06-008. United States Environmental Protection Agency, Washington, DC, USA.

- USEPA (2007) Simazine, Pesticide Tolerance. Federal Register, Docket # [EPA–HQ–OPP–2007–0187; FRL–8147–5, 72 (No. 181), 53449-53455. URL < https://www.gpo.gov/fdsys/pkg/FR-2007-09-19/pdf/E7-18508.pdf >
- USEPA (2012) Index to Pesticide Chemical Names, Part 180 Tolerance Information, and Food and Feed Commodities (by Commodity). United States Environmental Protection Agency, Office of Pesticide Programs, Washington, DC, USA. URL < https://www.epa.gov/sites/production/files/2015-01/documents/tolerances-commodity.pdf>
- USEPA (2014) Aquatic Life Benchmarks for Pesticide Registration. URL http://www2.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration
- USEPA (2015) Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.
- USFDA (2000) Industry Activities Staff Booklet. URL http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm">http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm"
 http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm"
 http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm"
 http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm"
 http://www.fda.gov/Food/GuidanceRegulatoryInformation/ChemicalContaminantsMetalsNaturalContaminantsMetalsNaturalContaminantsMetalsNaturalContaminantsMetalsNaturalContaminantsMetal
- Velisek J, Stara A, Machova J, Dvorak P, Zuskova E and Svobodova Z. (2012) Effects of low-concentrations of simazine on early life stages of common carp (*Cyprinus carpio* L.). *Neuroendocrinology Letters*, 33, 90-95.
- Velisek, J. (2013) Acute toxicity of triazine pesticides to juvenile signal crayfish (Pacifastacus leniusculus). Neuroendrocrinology Letters. 34, 31-36.
- Vervliet-Scheebaum M, Straus A, Tremp H, Hamer M, Maund SJ, Wagner E, and Schulz R. (2010) A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes. Environmental pollution, 158(2), 615-623.
- Wilkinson A D, Collier C J, Flores F and Negri AP. (2015). Acute and additive toxicity of ten photosystem-II herbicides to seagrass. *Scientific reports*, 5.
- Wilson PC, Whitwell T and Klaine SJ. (2001) Simazine toxicity and uptake by parrotfeather. *Journal of Aquatic Plant Management*, 39, 112-116.
- Wilson PC and Wilson SB. (2010) Toxicity of the herbicides bromacil and simazine to the aquatic macrophyte, Vallisneria americana Michx. Environmental Toxicology and Chemistry, 29(1), 201-211.
- Woodward Research Corporation. No date. Simazine, acute toxicity in goldfish. EPA MRID 23322.
- Worthing C, Hance R. (1990) *The Pesticide Manual 9th Edition*. British Crop Protection Council.
- Wu, Y.S., Lee, H.K. and Li, S.F., 1998. Rapid estimation of octanol-water partition coefficients of pesticides by micellar electrokinetic chromatography. *Electrophoresis*, 19(10), 1719-1727.

Data Tables

Table 3 Supplemental acute data rated RL, LR, LL.

Rating and exclusion reasons given. S: static; SR: static renewal; FT: flow-through. NR: not reported. 95% CI: 95% confidence interval. Exclusion reasons are listed at the end of the table.

Species	Common Identifier	Family	Test type	Meas/ Nom	Chemical grade	Duration	Temp (°C)	Endpoint	Age/ size	LC/EC ₅₀ (μg/L) (95% CI)	Reference	Rating/ Reason
Cyprinodon variegatus	Sheepshead minnnow	Cyprinodontidae	FT	Meas	96.90%	96-h	22	Mortality	0.36 g, 22 mm	> 4,300	Murphy 1992	1, 2
Dapnia magna	Daphnid	Daphniidae	S	Nom	96.00%	48-h	21	Immobilization	< 24-h	> 3,500	Marchini 1988	2, 3

Exclusion Reasons

- 1. Saltwater
- 2. Toxicity value not calculable
- 3. Control response low or not reported

Table 4 Final chronic plant toxicity data set for simazine.

All studies were rated RR. S: static; SR: static renewal. NR: not reported, n/a: not applicable. SMCV is in bold.

Species	Common identifier	Test type	Meas / Nom	Chemical grade	Duration	Temp (°C)	Endpoint	Age/ size	NOEC (μg/L)	LOEC (µg/L)	MATC (μg/L)	EC ₅₀ (μg/L)	Reference
Anabena flos- aquae	Cyano- bacterium	S	Meas	96.90%	5-d	24	Growth rate	Algal cells	20	38	28	98 (78- 17)	Swigert 1992a
Lemna minor	Duckweed	S	Nom	Technical	96-h	25	Growth inhibition (biomass)	NR	75	150	106	166 (102- 230)	Fairchild 1997
							Growth inhibition (number of plants, number of fronds), Growth					320 (230-	Thompson
Lemna gibba	Duckweed	SR	Meas	Technial	14-d	25	rate	2-w	54	110	77	430)	1992a
Navicula pelliculosa	Diatom	S	Meas	96.90%	5-d	20	Growth rate	Algal cells	33	66	47	300 (250- 440)	Thompson 1992b
Raphidocelis subcapitata	Alga	S	Meas	96.90%	5-d	24	Growth rate	Algal cells	68	130	94	260 (250- 270)	Thompson & Swigert 1992

Table 5 Acceptable reduced chronic data rated RR.

Reason for exclusion given below. S: static; SR: static renewal; FT: flow-through. NR: not reported

													Reason
	Common	Test	Meas/	Chemical		Temp		Age/	NOEC	LOEC	MATC		for
Species	identifier	type	Nom	grade	Duration	(°C)	Endpoint	size	(µg/L)	$(\mu g/L)$	$(\mu g/L)$	Reference	exclusion
Raphidocelis								Algal				Fairchild	_
subcapitata	Alga	S	Nom	Technical	96-h	25	Biomass	cells	600	1200	848.5	1997	A

A. Data calculated from nominal concentrations

Table 6 Supplemental chronic plant toxicity data for studies rated RL, LR, or LL.

S: static; SR: static renewal; FT: flow-through. NR: not reported, n/a: not applicable; 95% CI: 95% confidence interval; SE: standard error.

Species	Common identifier	Test type	Meas/ Nom	Chemical grade	Duration	Temp (°C)	Endpoint	Age/ size	NOEC (µg/L)	LOEC (µg/L)	EC ₅₀ (μg/L)	Reference	Rating/ Reason for exclusion
Anabena flos- aquae	Cyanobacterium	S	Nom	98.00%	96-h	24	Growth	Algal cells	NR	NR	71.8	Ma 2010	1, 2
Chlorella pyrenoidosa	Alga	S	Nom	92.20%	96-h	25	Growth	Algal cells	NR	NR	82	Ma 2002	1, 2
Chlorella vulgaris	Alga	S	Nom	92.20%	96-h	25	Growth	Algal cells	NR	NR	2173.8	Ma et al. 2002	1., 2
Microcystis aerunginosa	Alga	S	Nom	98.00%	96-h	24	Growth	Algal cells	NR	NR	304	Ma 2010	1, 2
Myriophyllum aqauticum	Parrotfeather	S	Nom	99.60%	7-d	25	Growth	NR	100	300	173	Wilson 2001	1, 2
Microcystis flos-aquae	Cyanobacterium	S	Nom	98.00%	96-h	24	Growth	Algal cells	NR	NR	110	Ma 2010	1, 2
Raphidocelis subcapitata	Alga	S	Nom	92.00%	96-h	25	Growth	Algal cells	NR	NR	748.5	Ma 2006	1, 2
Scenedesmus obliquus	Alga	S	Nom	92.20%	96-h	25	Growth	Algal cells	NR	NR	257	Ma 2002	1, 2
Scenedesmus quadricauda	Alga	S	Nom	92.20%	96-h	4*	Growth	Algal cells	NR	NR	150	Ma 2003	1, 2
Skeletonema costatum	Diatom	SR	Meas	96.50%	5-d	20	Growth rate	NR	250	NR	NR	Thompson 1992c	3

^{*}Low temperature suspected to be clerical error in publication

Exclusion Reasons

- 1. Not a standard method
- 2. Control not described and/or response not reported
- 3. Saltwater

Table 7 Supplemental chronic animal toxicity data for studies rated RL, LR, or LL.

S: static; SR: static renewal; FT: flow-through. NR: not reported; 95% CI: 95% confidence interval.

	Test	Meas	Chemical		Temp		Age/	NOEC	LOEC	MATC		Rating/ Reason for
Species	type	/Nom	grade	Duration	(°C)	Endpoint	size	(µg/L)	(μg/L)	(μg/L)	Reference	exclusion
-			3			•		G1	G1	4.6		
								survival:	survival:			
								1170	>1170			
								G2 10 d	G2 10 d			
								survival:	survival:			
								1170	>1170			
								Male	Male			
								body	body			
								length 14	length 14			
								d: 1170	d: >1170			
								Male	Male	Male		
								body	body	body		
								length 28	length 28	length 28		
								d: 319	d: 608	d: 440		
								Female	Female	Female		
								body	body	body		
								length 28	length 28	length 28		
						Survival,		d: 608	d: 1170	d: 843		
						Growth (body		Time to	Time to	Time to		
						length),		first	first	first		
Americamysis						Reproduction,		brood:	brood:	brood:	Lehman	
bahia	FT	Meas	96.90%	28-d	25	Time to brood	<24-h	319	608	440	2010	1
						Mortality,						
Cyprinus						Growth,					Velisek	
carpio	SR	Nom	99.50%	36-d	20	Histopathology	Eggs	0.06*	60*	1.9*	2012	2, 3*
						Mortality,					Plhalova	
Danio rerio	FT	Nom	99.50%	28-d	23	histopathology	20-d	6*	60*	19*	2010	3*

^{*}Based on histopathology

Exclusion Reasons

- 1. Saltwater
- 2. Control response not reported
- 3. Endpoint not linked to growth, reproduction or survival (Ch. 3, Section 3-2.1.3)

Table 8 Acceptable multispecies field, semi-field, laboratory, microcosm, mesocosm studies.

R= reliable; L= less reliable.

Reference	Habitat	Rating
	Fiberglass	
Vervliet-	tanks in	
Scheebaum	concrete	
et al. 2010	ponds	R
	In situ	_
Jenkins 1990	pond	L

Table 9 Threatened, Endangered, or Rare Species Predicted values by ICE.

Surr	ogate	Predicted LC ₅₀ (95% confidence	
G •	LC50	interval)	
Species	(µg/L)	Species	(μg/L)
Daphnia magna	3500	Daphnia pulex	2083.23 (128.62-33740.33)

Table 10 US EPA Aquatic Life Benchmarks.						
All units are μg/L. NR: not reported. (USEPA 2014)						
Acute Fish	Acute Fish Chronic Fish Acute Chronic Acute					
	Invertebrates Invertebrates nonvascular					
plants						
3200	NR	500	2.24	140		

Appendix A - Fit Test Calculations

MATCs used	Simazine all SMAVs 28 47 77 94	Omit one 1 47 77 94	2 28 77 94	3 28 47	28 47 77	5 28 47 77 94
	106	106	106	106	106	, ,
Omitted no	int vie	28	47	77	94	106
Omitted po	omi, XI;	20	4/	11	94	100
median 5th percentile Log-logistic		38.936	21.234	18.18	18.71	19.632
percentile		1.27	28.16	64.71	78.68	86.16
F-i(xi)		0.0127	0.2816	0.6471	0.7868	0.8616
1-F(xi)		0.9873	0.7184	0.3529	0.2132	0.1384
Min of F-i(x F(xi) p _i =2(min)	xi) or 1-	0.0127 0.0254	0.2816 0.5632	0.3529 0.7058	0.2132 0.4264	0.1384 0.2768

		Fisher test statistic		
		-2*Sum of		
p_i	$ln(p_i)$	ln (pi)	X^2_{2n}	
	-			$X^2 > 0.05$ so the distribution fits the simazine
0.0254	3.6730	13.4648	0.1988	chronic data set
	-			
0.5632	0.5741			
	-			
0.7058	0.3484			if $X^2 < 0.05$
	-			
0.4264	0.8524			if $X^2 > 0.05$

Appendix B - Aqueous Toxicity Data Summaries

Appendix B1 - Aqueous Toxicity Studies Rated RR

Anabena flos-aquae

Study: Swigert JP. (1992) A 5-day toxicity test with the freshwater alga (*Anabena flos-aquae*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-139. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42662401.

RelevanceReliabilityScore: 100Score: 95Rating: RRating: R

Relevance points taken off for: none.

Swigert 1992	A. flos-aquae
Value	Comment
Pesticide Assessment	
Guidelines, Subdivision J,	
Hazard Evaluation: Non-	
target Plants; Short-term	
Methods for Estimating the	
Chronic Toxicity of	
Effluents and Receiving	
Waters to Freshwater	
Organisms; 40CFR:	
Freshwater and Marine	
Algae Acute Toxicity Test;	
ASTM, Standard Guide for	
Conducting static 96-hour	
Toxicity tests with	
Microalgae	
Cyanobacteria	
Nostocales	
Nostocaceae	
Anabena	
Flos-aquae (Lyng.) Breb.	
Yes	
Exponential growth phase	
Laboratory cultures	
No	
Yes	
Not reported	Given organism
	size and presence in
	growth medium, it
	is assumed that
	aliquots are
	Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Non- target Plants; Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; 40CFR: Freshwater and Marine Algae Acute Toxicity Test; ASTM, Standard Guide for Conducting static 96-hour Toxicity tests with Microalgae Cyanobacteria Nostocales Nostocaceae Anabena Flos-aquae (Lyng.) Breb. Yes Exponential growth phase Laboratory cultures No Yes

	Swigert 1992	A. flos-aquae
Parameter	Value	Comment
		inherently randomly
Test vessels randomized?	Not reported	
Test duration	5 d	
Data for multiple times?	No	
Effect 1	Growth rate	
Control response 1	0.3395	
Temperature	24.3 ± 1.1 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/2153 lux	
Dilution water	Freshwater growth medium	
рН	Not reported	
Feeding	Growth medium	
Purity of test substance	96.9 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	100-113 %	
Toxicity values calculated based on	Measured	
nominal or measured		
concentrations?		
Chemical method documented?	GC	
Concentration of carrier (if any) in	0.12 mL/L dimethyl	
test solutions	formamide	
Concentration 1 Nom; Meas (µg/L)	19; 20	$3 \text{ reps}, 1.0 \times 10^4$
		cells/rep
Concentration 2 Nom; Meas (µg/L)	38; 38	$3 \text{ reps}, 1.0 \times 10^4$
		cells/rep
Concentration 3 Nom; Meas (µg/L)	75; 78	3 reps, 1.0 x 10 ⁴
		cells/rep
Concentration 4 Nom; Meas (µg/L)	150; 170	$3 \text{ reps}, 1.0 \times 10^4$
		cells/rep
Concentration 5 Nom; Meas (µg/L)	300; 320	3 reps, 1.0 x 10 ⁴
		cells/rep
Concentration 6 Nom; Meas (µg/L)	600; 660	$3 \text{ reps}, 1.0 \times 10^4$
		cells/rep
Control	Solvent: 0; 0	$3 \text{ reps}, 1.0 \times 10^4$
	Negative: 0; 0	cells/rep
EC ₅₀ (95% CI) (μg/L)	98 (78-17)	Method: binomial
NOEC	20	Method: binomial
		p: not reported
		MSD: not reported
LOEC	38	0.2120 (1.2)
% control at NOEC	92 %	0.3130 (tmt) /
		0.3395 (mean
0, 1,7070	01.04	controls) = 92
% control at LOEC	81 %	0.2733 (tmt) /
		0.3395 (mean
		controls) = 81

Notes:

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires distilled water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

Documentation: Minimum significant difference (2. Total: 100- 2=98

<u>Acceptability:</u> Carrier solvent (4), Random design (2), Minimum significant difference (1), % control at LOEC (1). Total: 100-8 =92

Reliability score: mean(98, 92)=95

Lemna gibba

Study: Thompson, SG. 1992a. Simazine a 14-day toxicity test with duckweed (*Lemna Gibba G3*). Wildlife International, Easton, Maryland. Laboratory study number 108A-137. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 4253704. CA DPR 138090.

RelevanceReliabilityScore: 100Score: 93Rating: RRating: R

Relevance points taken off for: none.

	Thompson 1992a	L. gibba
Parameter	Value	Comment
Test method cited	Pesicide Assessment	
	Guidelines, Subdivision J	
	Hazard Evaluation:	
	Nontarget Plants and ASTM	
	Standard E 1415-91	
Order	Alismatales	
Family	Araceae	
Genus	Lemna	
Species	gibba	
Family native to North America?	Yes	
Age/size at start of test/growth	2 w	
phase		
Source of organisms	Laboratory cultures	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Yes	
Test duration	14 d	
Data for multiple times?	3, 6, 9, 12, 14	
Effect 1	Number of plants	
Control response 1	Negative: 205	
	Solvent: 257	
Effect 2	Number of fronds	
Control response 2	Negative: 698	
	Solvent: 848	
Effect 3	Growth rate	
Control response 3, mean controls	0.1194 / day	
Temperature	25 ± 2 °C	
Test type	Static renewal	
Photoperiod/light intensity	Continuous; 6458 lux	

	Thompson 1992a	L. gibba
Parameter	Value	Comment
Dilution water	Growth medium	M-Hoagland
		without EDTA or
		sucrose made with
		deionized well
		water
рН	Not reported	
Feeding	Growth medium	
Purity of test substance	Technical grade	Label: 96.9 %
Concentrations measured?	Yes	
Measured is what % of nominal?	102-120%	
Toxicity values calculated based on	Measured	
nominal or measured		
concentrations?		
Chemical method documented?	GC	
Concentration of carrier (if any) in	Dimethyl formamide, 150	
test solutions	μg/L	17.10
Concentration 1 Nom; Meas (µg/L)	25; 27	reps, 15-18
	50.54	fronds/rep
Concentration 2 Nom; Meas (µg/L)	50; 54	
Concentration 3 Nom; Meas (µg/L)	100; 110	
Concentration 4 Nom; Meas (µg/L)	200; 230	
Concentration 5 Nom; Meas (µg/L)	400; 430	
Concentration 6 Nom; Meas (µg/L)	800; 880	
Control	Negative: 0; 0	
	Solvent: 0; 0	
IC ₅₀ (95% CI) (μg/L)	320 (230-430)	Method: binomial
		probability
NOEC	54 μg/L	Method:
		p:
		MSD:
LOEC	110 μg/L	
MATC (GeoMean NOEC, LOEC)	77	
% control at NOEC	Number of plants:	Number of plants:
70 COMITOT AT NOTEC	3 d: 117 %	3d: 7 (tmt) / 6
	6 d: 90 %	(mean control) =
	9 d: 110 %	117
	12 d: 99 %	6d: 30 (tmt) / 33.5
	14 d: 87 %	(mean control) = 90
		9d: 64 (tmt) / 58
	Number of fronds:	(mean control) =
	3 d: 104 %	110
	6 d: 92 %	12d: 141 (tmt) / 143
	9 d: 103 %	(mean control) = 99
	12 d: 99 %	14d: 200 (tmt) / 231
	14 d: 91 %	(mean control) = 87

	Thompson 1992a	L. gibba
Parameter	Value	Comment
% control at LOEC	Number of plants: 3 d: 100 % 6 d: 66 % 9 d: 72 % 12 d: 68 % 14 d: 54 % Number of fronds: 3 d: 91 % 6 d: 66 % 9 d: 72 % 12 d: 61 % 14 d: 61 %	Number of fronds: 3d: 48 (tmt) / 46 (mean control) = 6d: 126 (tmt) / 136.5 (mean control) = 9d: 300 (tmt) / 292.5 (mean control) = 12d: 141 (tmt) /143 (mean control) = 99 14d: 701 (tmt) / 773 (mean control) = 91 Number of plants: 3d: 6 (tmt) / 6 (mean control) = 100 6d: 22 (tmt) / 33.5 (mean control) = 66 9d: 42 (tmt) / 58 (mean control) = 72 12d: 97 (tmt) / 143 (mean control) = 68 14d: 125 (tmt) / 231 (mean control) = 54
		Number of fronds: 3d: 42 (tmt) / 46 (mean control) = 91 6d: 90 (tmt) / 136.5 (mean control) = 66 9d: 210 (tmt) / 292.5 (mean control) = 72 12d: 349 (tmt) / 569.5 (mean control) = 61 14d: 475 (tmt) / 773 (mean control) = 61

Notes: $IC_{50} = 50\%$ reduction in growth rate.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires distilled water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Simazine solubility (S) = 5,450 μ g/L, 2S = 10, 900 μ g/L. All exposure concentrations were acceptable.

Reliability points taken off for:

<u>Documentation:</u> Statistical significance (2), Significance level (2), Minimum significant difference (2). Total: 100-6 = 94

<u>Acceptability:</u> Organisms randomized (1), Temperature variation (3), Hypothesis tests (3), Minimum significant difference (1). Total: 100- 8=92

Reliability score: mean(94,92)=93

Lemna minor

Study: Fairchild, J.F., Ruessler, D.S., Haverland, P.S. and Carlson, A.R., 1997. Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides. Archives of Environmental Contamination and Toxicology, 32(4), 353-357.

RelevanceReliabilityScore: 92.5Score: 75Rating: RRating: R

Relevance points taken off for: Control response (7.5). 100-7.5=92.5

	Fairchild et al. 1997	L. minor
Parameter	Value	Comment
Test method cited	American Society for	
	Testing and Materials. 1993.	
	Standard guide for	
	conducting static 96h	
	toxicity tests with	
	microalgae: Practice E	
	1218-90. In: Annual book of	
	ASTM standards:Water and	
	environmental	
	technology. ASTM	
	Committee E-47 on	
	Biological Effects	
	and Environmental Fate,	
	American Society for	
	Testing and Materials,	
	Philadelphia, PA, p 929	
Order	Alismatales	
Family	Araceae	
Genus	Lemna	
Species	minor	
Family native to North America?	Yes	
Age/size at start of test/growth	Not reported	
phase		
Source of organisms	Carolina Biological Supply	Burlington, North
	Company	Carolina
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Yes	
Test duration	96 h	

	Fairchild et al. 1997	L. minor
Parameter	Value	Comment
Data for multiple times?	48, 72, 96 h	
Effect 1	Biomass	
Control response 1	Not reported	
Temperature	25 °C	
Test type	Static	
Photoperiod/light intensity	16:8 light:dark/400 foot- candle	
Dilution water	Nutrient enriched water, modified from APHA 1985	American Public Health Association, American Water Works Association, and the Water Pollution Control Federation (1985) Standard methods for the examination of water and wastewater, 14th ed., APHA-AWWA- WPCF, Washington, DC.
Feeding	Nutrient enriched water	
Purity of test substance	Technical	
Concentrations measured?	No	
Measured is what % of nominal?	Not applicable	
Toxicity values calculated based on nominal or measured concentrations?	Nominal	
Chemical method documented?	Not applicable	
Concentration of carrier (if any) in test solutions	Acetone, concentration not reported	
Concentration 1 Nom; Meas (µg/L)	Concentrations not reported, 5 concentrations plus solvent and negative controls	3 reps, 12 fronds/rep
Control	Solvent Negative	
EC ₅₀ (95% CI) (μg/L)	166 (102-230)	Method: nonlinear regression
NOEC	75	Method: Duncan's Multiple Range Test p: 0.05 MSD: not reported

	Fairchild et al. 1997	L. minor
Parameter	Value	Comment
LOEC	150	
MATC (GeoMean NOEC, LOEC)	106	
% control at NOEC	Not calculable	
% control at LOEC	Not calculable	

Notes: Raw data not reported so % controls at NOEC/LOEC not calculable and control responses unknown.

Simazine solubility (S) = 5,450 μ g/L, 2S = 10, 900 μ g/L.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because the nutrient enriched water used is an industry standard and the medium is presumably appropriate for the test species because a specific water was used.

Reliability points taken off for:

<u>Documentation</u>: Organism life stage/size (5), Nominal concentrations (3), Measured concentrations (3), Statistical significance (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-17 =83

Acceptability: Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Carrier solvent (4), Temperature variation (3), Number of concentrations (3), Dilution factor (2), Hypothesis tests (3). Total: 100- 32=68

Reliability score: mean(83,67)=75

Navicula pelliculosa

Study: Thompson SG. (1992b) A 5-day toxicity test with the freshwater alga (*Navicula pelliculosa*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-138. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503707. CA DPR 138087.

RelevanceReliabilityScore: 100Score: 92Rating: RRating: R

	Thompson 1992b	N. pelliculosa
Parameter	Value	Comment
Test method cited	Pesticide Assessment	
	Guidelines, Subdivision J,	
	Hazard Evaluation: Non-	
	target Plants; Short-term	
	Methods for Estimating the	
	Chronic Toxicity of	
	Effluents and Receiving	
	Waters to Freshwater	
	Organisms; 40CFR:	
	Freshwater and Marine	
	Algae Acute Toxicity Test;	
	ASTM, Standard Guide for	
	Conducting static 96-hour	
	Toxicity tests with	
	Microalgae	
Division	Heterokontophyta	
Class	Bacillariophyceae	
Order	Naviculales	
Family	Naviculaceae	
Genus	Navicula	
Species	pelliculosa	
Family native to North America?	Yes	
Age/size at start of test/growth	Exponential growth phase	
phase		
Source of organisms	Laboratory cultures	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	Given organism
		size and presence in
		growth medium, it
		is assumed that

	Thompson 1992b	N. pelliculosa
Parameter	Value	Comment
		aliquots are
		inherently randomly
Test vessels randomized?	Not reported	
Test duration	5 d	
Data for multiple times?	No	
Effect 1	Growth rate	
Control response 1	0.4699	
Temperature	20.6 ± 0.4 °C	
Test type	Static	
Photoperiod/light intensity	16:8/4306 lux	
Dilution water	Freshwater growth medium	Deionized well water
рН	7.2-8.4	
Feeding	Growth medium	
Purity of test substance	96.9 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	78-108 %	
Toxicity values calculated based on	Measured	
nominal or measured		
concentrations?		
Chemical method documented?	GC	
Concentration of carrier (if any) in	0.2 mL/L	
test solutions		
Concentration 1 Nom; Meas (µg/L)	31; 33	$3 \text{ reps}, 1.0 \times 10^4$
		cells/rep
Concentration 2 Nom; Meas (µg/L)	63; 66	3 reps, 1.0 x 10 ⁴
		cells/rep
Concentration 3 Nom; Meas (µg/L)	125; 130	$3 \text{ reps}, 1.0 \times 10^4$
		cells/rep
Concentration 4 Nom; Meas (µg/L)	250; 250	$3 \text{ reps}, 1.0 \times 10^4$
	700 440	cells/rep
Concentration 5 Nom; Meas (µg/L)	500; 440	3 reps, 1.0×10^4
	1000, 940	cells/rep
Concentration 6 Nom; Meas (µg/L)	1000; 840	3 reps, 1.0 x 10 ⁴ cells/rep
Control	Negative: 0; 0	3 reps, 1.0 x 10 ⁴
Condoi	Solvent: 0; 0	cells/rep
EC ₅₀ (95% CI) (μg/L)	300 (250-440)	Method: binomial
NOEC	33	Method: binomial
TOLC		p: not reported
		MSD: not reported
LOEC	66	
% control at NOEC	99 %	0.4643 (tmt) /
		0.4699 (mean
		controls) = 99
% control at LOEC	89 %	0.4233 (tmt) /

	Thompson 1992b	N. pelliculosa
Parameter	Value	Comment
		0.4699 (mean
		controls) = 89

Notes:

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires deionized water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation:</u> Significance level (2), Minimum significant difference (2). Total: 100-4 = 96

<u>Acceptability:</u> Measured concentrations within 20% nominal (4), Carrier solvent (4), Random design (2), Minimum significant difference (1), % control at LOEC (1). Total: 100- 12=88

Reliability score: mean(96,88)=92

Raphidocelis subcapitata

Study: Fairchild, J.F., Ruessler, D.S., Haverland, P.S. and Carlson, A.R., 1997. Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides. Archives of Environmental Contamination and Toxicology, 32(4), 353-357.

RelevanceReliabilityScore: 92.5Score: 75.5Rating: RRating: R

Relevance points taken off for: Control response (7.5). 100-7.5=92.5

	Fairchild et al. 1997	R. subcapitata
Parameter	Value	Comment
Test method cited	American Society for	
	Testing and Materials. 1993.	
	Standard guide for	
	conducting static 96h	
	toxicity tests with	
	microalgae: Practice E	
	1218-90. In: Annual book of	
	ASTM standards:Water and	
	environmental	
	technology. ASTM	
	Committee E-47 on	
	Biological Effects	
	and Environmental Fate,	
	American Society for	
	Testing and Materials,	
	Philadelphia, PA, p 929	
Phylum/subphylum	Chlorophyta	
Class	Chlorophyceae	
Order	Sphaeropleales	
Family	Selenastraceae	
Genus	Raphidocelis	
Species	subcapitata	
Family native to North America?	Yes	
Age/size at start of test/growth	Not reported	
phase		
Source of organisms	Carolina Biological Supply	Burlington, North
	Company	Carolina
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	Given organism

	Fairchild et al. 1997	R. subcapitata
Parameter	Value	Comment
		size and presence in
		growth medium, it
		is assumed that
		aliquots are
		inherently randomly
Test vessels randomized?	Yes	
Test duration	96 h	
Data for multiple times?	48, 72, 96 h	
Effect 1	Biomass	
Control response 1	Not reported	
Temperature	25 °C	
Test type	Static	
Photoperiod/light intensity	16:8 light:dark/400 foot-	
	candle	
Dilution water	ASTM growth medium	
Feeding	Growth medium	
Purity of test substance	Technical	
Concentrations measured?	No	
Measured is what % of nominal?	Not applicable	
Toxicity values calculated based on	Nominal	
nominal or measured		
concentrations?		
Chemical method documented?	Not applicable	
Concentration of carrier (if any) in	Acetone, concentration not	
test solutions	reported	
Concentration 1 Nom; Meas (µg/L)	Concentrations not reported,	3 reps, 20,000
, , ,	5 concentrations plus	cells/mL/rep
	solvent and negative	
	controls	
Control	Solvent	
	Negative	
EC ₅₀ (95% CI) (μg/L)	1240 (1088-1393)	Method: nonlinear
		regression
NOEC	600	Method: Duncan's
		Multiple Range
		Test
		p: 0.05
		MSD: not reported
LOEC	1200	
MATC (GeoMean NOEC, LOEC)	848.5	
% control at NOEC	Not calculable	
% control at LOEC	Not calculable	
Notes: Pow date not reported so % or	· 1 · NOEG/LOEG · 1	111 1 . 1

Notes: Raw data not reported so % controls at NOEC/LOEC not calculable and control responses unknown.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used is an ASTM standard for this species, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation:</u> Organism life stage/size (5), Nominal concentrations (3), Measured concentrations (3), Statistical significance (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-17 =83

<u>Acceptability:</u> Control response (9),Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Carrier solvent (4), Temperature variation (3), Number of concentrations (3), Dilution factor (2), Hypothesis tests (3). Total: 100- 32=68

Reliability score: mean(83,68)=75.5

Raphidocelis subcaptitata

Study: Thompson, SG and Swigert, JP. (1992) A 5-day toxicity test with the freshwater alga (*Selenastrum capricornum*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-141. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503706. CA DPR 138086.

RelevanceReliabilityScore: 100Score: 94.5Rating: RRating: R

	Thompson & Swigert 1992	R. subcapitata
Parameter	Value	Comment
Test method cited	Pesticide Assessment	
	Guidelines, Subdivision J,	
	Hazard Evaluation: Non-	
	target Plants; Short-term	
	Methods for Estimating the	
	Chronic Toxicity of	
	Effluents and Receiving	
	Waters to Freshwater	
	Organisms; 40CFR:	
	Freshwater and Marine	
	Algae Acute Toxicity Test;	
	ASTM, Standard Guide for	
	Conducting static 96-hour	
	Toxicity tests with	
	Microalgae	
Phylum/subphylum	Chlorophyta	
Class	Chlorophyceae	
Order	Sphaeropleales	
Family	Selenastraceae	
Genus	Raphidocelis	
Species	subcapitata	
Family native to North America?	Yes	
Age/size at start of test/growth	Exponential growth phase	
phase		
Source of organisms	Laboratory cultures	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	Given organism
		size and presence in
		growth medium, it
		is assumed that

	Thompson & Swigert 1992	R. subcapitata
Parameter	Value	Comment
		aliquots are
		inherently randomly
Test vessels randomized?	Not reported	
Test duration	5 d	
Data for multiple times?	No	
Effect 1	Growth rate	
Control response 1	Mean: 0.5374	
Temperature	23.8 ± 0.4 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/ 4306 lux	
Dilution water	Freshwater growth medium	Made with dionized well water
рН	7.3-8.2	
Hardness	mg/L CaCO ₃	
Alkalinity	mg/L CaCO ₃	
Conductivity	umhos/cm	
Dissolved Oxygen	mg/L	
Feeding	Growth medium	
Purity of test substance	96.9 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	100-116 %	
Toxicity values calculated based on nominal or measured concentrations?	Measured	
Chemical method documented?	GC	
Concentration of carrier (if any) in	0.2 mL/L dimethyl	
test solutions	formamide	
	31; 34	3 reps, 1.0x 10 ⁴
Concentration 1 Nom; Meas (μg/L)	31, 34	cells/rep
Concentration 2 Nom; Meas (µg/L)	63; 68	3 reps, 1.0 x 10 ⁴ cells/rep
Concentration 3 Nom; Meas (µg/L)	125; 130	3 reps, 1.0 x 10 ⁴ cells/rep
Concentration 4 Nom; Meas (µg/L)	250; 290	3 reps, 1.0 x 10 ⁴ cells/rep
Concentration 5 Nom; Meas (µg/L)	500; 540	3 reps, 1.0 x 10 ⁴ cells/rep
Concentration 6 Nom; Meas (µg/L)	1000; 1000	3 reps, 1.0 x 10 ⁴ cells/rep
Control	Solvent: 0; 0	3 reps, 1.0 x 10 ⁴
77 (07) (7)	Negative: 0; 0	cells/rep
EC ₅₀ (95% CI) (μg/L)	260 (250-270)	Method: moving
NOEC	68	average Method: moving
		average

	Thompson & Swigert 1992	R. subcapitata
Parameter	Value	Comment
		p: not reported
		MSD: not reported
LOEC	130	
% control at NOEC	96 %	0.5178 (tmt) /
		0.5374 (mean
		controls) = 96
% control at LOEC	85 %	0.4594 (tmt) /
		0.5374 (mean
		controls) = 85

Notes:

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires deionized water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

Documentation: Significance level (2), Minimum significant difference (2). Total: 100-4 = 96

<u>Acceptability:</u> Carrier solvent (4), Random design (2), Minimum significant difference (1), % control at NOEC (1). Total: 100-7=93

Reliability score: mean(96, 93)=94.5

Appendix B2 - Wildlife Toxicity Studies Rated R

Anas platyrhynchos

Study: Beavers JB, Foster JW, Mitchell LR, Jaber M. 1994. A reproduction study with the mallard. Wildlife International, Ltd., Easton, Maryland. Wildlife International, Ltd. project number 108-356. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. CA DPR 139747.

Documentation and acceptability rating for terrestrial laboratory/field data (adapted from ECOTOX 2006). Score is given if parameter is reported.

Parameter ¹	Score ²	Points
Exposure duration	20	20
Control type	7	7
Organism information (i.e., age, life stage)	8	8
Chemical grade or purity	5	5
Chemical analysis method	5	5
Exposure type (i.e., dermal, dietary, gavage)	10	10
Test location (i.e., laboratory, field, natural artificial)	5	5
Application frequency	5	5
Organism source	5	5
Organism number and/or sample number	5	5
Dose number	5	5
Statistics		
Hypothesis tests		
Statistical significance	5	5
Significance level	5	5
Minimum significant difference	3	0
% of control at NOEC and/or LOEC	3	3
Point estimates (i.e., LC ₅₀ , EC ₅₀)	4	0
Total	100	93

¹ Compiled from RIVM (2001), USEPA (1985; 2003b), ECOTOX (2006), CCME (1999), ANZECC & ARMCANZ (2000), OECD (1995), and Van Der Hoeven *et al.* (1997).

² Weighting based acceptability criteria from various ASTM, OECD, APHA, and USEPA methods, ECOTOX (2006), and on data quality criteria in RIVM (2001), USEPA (1985; 2003b), CCME (1999), ANZECC & ARMCANZ (2000), OECD (1995), and Van Der Hoeven *et al.* (1997).

Anas platyrhynchos

Rieder D (reviewer). (1965) Simazine, subacute toxicity in mallard ducks. Truslo Farm Inc., Easton, Maryland. Later became Wildlife International (laboratory). USEPA MRID 43672.

Documentation and acceptability rating for terrestrial laboratory/field data (adapted from ECOTOX 2006). Score is given if parameter is reported.

Parameter ¹	Score ²	Points
Exposure duration	20	20
Control type	7	7
Organism information (i.e., age, life stage)	8	8
Chemical grade or purity	5	5
Chemical analysis method	5	0
Exposure type (i.e., dermal, dietary, gavage)	10	10
Test location (i.e., laboratory, field, natural artificial)	5	5
Application frequency	5	5
Organism source	5	5
Organism number and/or sample number	5	5
Dose number	5	5
Statistics		
Hypothesis tests		
Statistical significance	5	0
Significance level	5	0
Minimum significant difference	3	0
% of control at NOEC and/or LOEC	3	3
Point estimates (i.e., LC ₅₀ , EC ₅₀)	4	4
Total	100	82

¹ Compiled from RIVM (2001), USEPA (1985; 2003b), ECOTOX (2006), CCME (1999), ANZECC & ARMCANZ (2000), OECD (1995), and Van Der Hoeven *et al.* (1997).

² Weighting based acceptability criteria from various ASTM, OECD, APHA, and USEPA methods, ECOTOX (2006), and on data quality criteria in RIVM (2001), USEPA (1985; 2003b), CCME (1999), ANZECC & ARMCANZ (2000), OECD (1995), and Van Der Hoeven *et al.* (1997).

Anas platyrhynchos

Rieder D (reviewer). (1974) One-generation reproduction study-mallard ducks. Truslo Farm Inc., Easton, Maryland. Later became Wildlife International (laboratory). Project number 108-101. Submitted to -Geigy Corporation, Greensboro, NC. USEPA MRID 43678.

No adverse affects at tested concentrations so it can be stated that $EC_{50} > 20$ mg/kg.

Documentation and acceptability rating for terrestrial laboratory/field data (adapted from ECOTOX 2006). Score is given if parameter is reported.

Parameter ¹	Score ²	Points
Exposure duration	20	20
Control type	7	7
Organism information (i.e., age, life stage)	8	8
Chemical grade or purity	5	5
Chemical analysis method	5	
Exposure type (i.e., dermal, dietary, gavage)	10	10
Test location (i.e., laboratory, field, natural artificial)	5	5
Application frequency	5	5
Organism source	5	5
Organism number and/or sample number	5	5
Dose number Two concentrations plus control (2 and 20	5	0
mg/kg)		
Statistics		
Hypothesis tests		
Statistical significance	5	5
Significance level	5	5
Minimum significant difference	3	0
% of control at NOEC and/or LOEC	3	0
Point estimates (i.e., LC ₅₀ , EC ₅₀) No adverse affects	4	0
Total	100	85

¹ Compiled from RIVM (2001), USEPA (1985; 2003b), ECOTOX (2006), CCME (1999), ANZECC & ARMCANZ (2000), OECD (1995), and Van Der Hoeven *et al.* (1997).

² Weighting based acceptability criteria from various ASTM, OECD, APHA, and USEPA methods, ECOTOX (2006), and on data quality criteria in RIVM (2001), USEPA (1985; 2003b), CCME (1999), ANZECC & ARMCANZ (2000), OECD (1995), and Van Der Hoeven *et al.* (1997).

Appendix B3 - Mesocosm studies rated R

Simazine

Elodea Canadensis Persicaria amphibian Myriophyllum spicatum Glyceria maxima

Vervliet-Scheebaum M, Straus A, Tremp H, Hamer M, Maund SJ, Wagner E, and Schulz R. (2010) A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes. *Environmental pollution*, 158(2), 615-623.

Documentation and acceptability (reliability) evaluation for data derived from aquatic outdoor field and indoor model ecosystems experiments. Include notes next to each parameter. Adapted from ECOTOX 2006; Table from TenBrook et al. 2010.

Parameter ^a	Scoreb	Points
Results published or in signed, dated format Published peer review article	5	5
Exposure duration and sample regime adequately described	6	6
Unimpacted site (Score 7 for artificial systems) fiberglass tanks inside concrete ponds		7
Adequate range of organisms in system (1° producers, 1°, 2° consumers) Rooted macrophytes (P. amphibia, E. canadensis, M. spicatum, and G. maxima) and naturally occurring alga		6
Chemical		
Grade or purity stated formulation, GESATOP 500 FW, 50% active ingredient	6	0
Concentrations measured/estimated and reported Nominal: 50, 500, 5000 μg/L; Measured: 80, 1100, 8500 μg/L	8	8
Analysis method stated LC-MS/MS	2	2
Habitat described (e.g., pond, lake, ditch, artificial, lentic, lotic) fiberglass tanks inside concrete ponds	6	6
Water quality		
Source identified Aged tap water	2	2
Hardness reported	1	0
Alkalinity reported	1	0
Dissolved oxygen reported Plotted, approximately 50-140%	2	2
Temperature reported 15-22°C, ambient	2	2
Conductivity reported Plotted, approximately 350-675 µS/cm	1	1
pH reported Plotted, approximately 7.5-9.75	1	1
Photoperiod reported	1	1
Organic carbon reported	2	0

Parameter ^a	Score ^b	Points
Chemical fate reported	3	3
Geographic location identified (Score 2 for indoor systems) 51°26'58"N, 0°44'58"W	2	2
Pesticide application		
Type reported (e.g., spray, dilutor, injection) stirred into water column	2	2
Frequency reported	2	2
Date/season reported (Score 2 for indoor systems) May-September 2004	2	2
Test endpoints		
Species abundance reported Figure 4	3	3
Species diversity reported *See note at end	3	0
Biomass reported Wet and dry weight of some macrophytes only	2	2
Ecosystem recovery reported	2	2
Statistics		
Methods identified Dunnett's test	2	2
At least 2 replicates 3 replicates	3	3
At least 2 test concentrations and 1 control 3 concentrations, 1 control	3	3
Dose-response relationship observed	2	2
Hypothesis tests		
NOEC determined Based on nominal concentration, length increase of main shoot 84 d: 50 $\mu g/L$	4	4
Significance level stated 0.05	2	2
Minimum significant difference reported	2	0
% of control at NOEC and/or LOEC reported or calculable Data in table 2	2	2
Total Reliability	100	85

 $\overline{\text{LOEC}}$ = lowest observed effect concentration, $\overline{\text{NOEC}}$ = no observed effect concentration.

^aCompiled from RIVM 2001, USEPA 1985 and 2003a, ECOTOX 2006, CCME 1995, ANZECC and ARMCANZ 2000, OECD 1995a, and van der Hoeven et al. 1997.

^bWeighting based on ECOTOX 2006 and on data quality criteria in RIVM 2001 and OECD 1995a.

^{*}Criteria for algae: "the species had to be present in the sample in all three replicates with at least 40.8 individuals per mL per microcosm or they had to be present in at least two out of three replicates per treatment with 204 individuals per mL. This procedure allowed for a reduction of the number of species evaluated and ensured that only those species that were present in high abundance were considered."

Appendix B4 – Studies rated RL, LR, LL

Americamysis bahia

Study: Lehman, C. 2010. Simazine-life-cycle toxicity with the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions. ABC Laboratories, Columbia, Missouri. Laboratory report number 65071. Submitted to Syngenta Crop Protection, Inc., Greensboro, North Carolina. EPA MRID 47984801.

RelevanceReliabilityScore: 85Score: 91Rating: LRating: R

Relevance points taken off for: Freshwater (15). 100-15=85

	Lehman 2010	A. bahia
Parameter	Value	Comment
Test method cited	US EPA Office of	
	Prevention, Pesticides, and	
	Toxic Substance Ecological	
	Effects Test Guideline	
	850.1350; ASTM standard	
	guide E1191; US EPA	
	FIFRA Subdivision E,	
	Section 72-4	
Phylum/subphylum	Arthropoda/Crustacea	
Class	Malacostraca	
Order	Mysida	
Family	Mysidae	
Genus	Americamysis	
Species	bahia	
Family native to North America?	Yes	
Age/size at start of test/growth	< 24 h	
phase		
Source of organisms	Laboratory cultures	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Yes	
Test duration	28 d	
Data for multiple times?	7, 14, 21, 28 d	
Effect 1	G ₁ survival	
Control response 1	28 d: 98 %	
Effect 2	G ₂ survival	
Control response 2	7 d: 98 %	
Effect 3	Adult body length	

	Lehman 2010	A. bahia
Parameter	Value	Comment
Control response 3	Male:	
	14 d: 5.23 mm	
	28 d: 6.21 mm	
	Female:	
	14 d: 5.37 mm	
	28 d: 6.45 mm	
Effect 4	Reproduction	Young per female
Control response 4	33.5	
Effect 5	Time to brood	
Control response 5	10 d	
Temperature	24.75 ± 0.75 °C	
Test type	Flow through	
Photoperiod/light intensity	141:10 d/561 lux	
Dilution water	Laboratory saltwater	Made with
		commercial sea salt
		mix and
		demineralized well
		water (salinity: 20
		‰)
Dissolved oxygen	5.26-7.85 mg/L	62-93 %
Feeding	Live brine shrimp nauplii	
	(Artemia sp.), 2/d	
Purity of test substance	96.9 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	63-80 %	
Toxicity values calculated based on	Measured	
nominal or measured		
concentrations?	1.0.160.10	
Chemical method documented?	LC-MS/MS	
Concentration of carrier (if any) in	Not used	
test solutions	100 62 2	2 15/
Concentration 1 Nom; Meas (µg/L)	100; 63.3	3 reps, 15/rep
Concentration 2 Nom; Meas (µg/L)	200; 151	3 reps, 15/rep
Concentration 3 Nom; Meas (µg/L)	400; 319	3 reps, 15/rep
Concentration 4 Nom; Meas (µg/L)	800; 608	3 reps, 15/rep
Concentration 5 Nom; Meas (µg/L)	1600; 1170	3 reps, 15/rep
Control	0; 0	3 reps, 15/rep
LC ₅₀ (95% CI) (μg/L)	G ₁ survival: >1170	Method: probit
	G ₂ 10 d survival: >1170	
NOEC	G ₁ survival: 1170	Method: ANOVA
	G ₂ 10 d survival: 1170	p: 0.05
	Male body length 14 d:	MSD: not reported
	1170	
	Male body length 28 d: 319	
	Female body length 28 d:	
	608	

$ \begin{array}{ c c c c c } \hline \textbf{Parameter} & \textbf{Value} & \textbf{Comment} \\ \hline & & & & & & & & & \\ \hline & & & & & & &$		an 2010	A. bahia
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	arameter		Comment
$\begin{array}{c} G_2 \ 10 \ d \ survival: > 1170 \\ Male \ body \ length \ 14 \ d: \\ > 1170 \\ Male \ body \ length \ 28 \ d: \ 608 \\ Female \ body \ length \ 28 \ d: \\ 1170 \\ Time \ to \ first \ brood: \ 608 \\ \hline MATC \ (GeoMean \ NOEC, \ LOEC) \\ Male \ body \ length \ 28 \ d: \ 440 \\ Female \ body \ length \ 28 \ d: \ 843 \\ Time \ to \ first \ brood: \ 440 \\ \hline \% \ \ control \ at \ NOEC \\ G_1 \ survival: \\ 7 \ d: \ 95 \\ 14 \ d: \ 95 \\ 21 \ d: \ 102 \\ \hline \end{array}$		to first brood: 319	
Male body length 14 d: >1170 Male body length 28 d: 608 Female body length 28 d: 1170 Time to first brood: 608 MATC (GeoMean NOEC, LOEC) Male body length 28 d: 440 Female body length 28 d: 843 Time to first brood: 440 W control at NOEC G ₁ survival: 7 d: 95 14 d: 95 21 d: 102 G ₁ survival 7 d: 93 (tmt) / 98 (control) = 95	DEC	vival: >1170	
>1170 Male body length 28 d: 608 Female body length 28 d: 1170 Time to first brood: 608 MATC (GeoMean NOEC, LOEC) Male body length 28 d: 440 Female body length 28 d: 843 Time to first brood: 440 Sq. survival: 7 d: 95 7 d: 93 (tmt) / 98 (control) = 95 14 d: 95 21 d: 102 Control 28 d: 608 Control Control		d survival: >1170	
$\begin{tabular}{lllllllllllllllllllllllllllllllllll$		oody length 14 d:	
Female body length 28 d: 1170 Time to first brood: 608 MATC (GeoMean NOEC, LOEC) Male body length 28 d: 440 Female body length 28 d: 843 Time to first brood: 440 % control at NOEC $G_1 \text{ survival:}$ $7 \text{ d: } 95$ $14 \text{ d: } 95$ $21 \text{ d: } 102$ Gianting to first brood: 450 Gianting to first brood: 440			
$\begin{array}{c c} & 1170 \\ & \text{Time to first brood: } 608 \\ \hline \text{MATC (GeoMean NOEC, LOEC)} & \text{Male body length } 28 \text{ d: } 440 \\ & \text{Female body length } 28 \text{ d: } \\ & 843 \\ & \text{Time to first brood: } 440 \\ \hline \text{\% control at NOEC} & G_1 \text{ survival: } \\ & 7 \text{ d: } 95 \\ & 14 \text{ d: } 95 \\ & 21 \text{ d: } 102 \\ \hline \end{array} \qquad \begin{array}{c} G_1 \text{ survival: } \\ & 7 \text{ d: } 93 \text{ (tmt) } / 98 \\ & (\text{control)} = 95 \\ \hline \end{array}$		oody length 28 d: 608	
$ \begin{array}{c c} & \text{Time to first brood: } 608 \\ \hline \text{MATC (GeoMean NOEC, LOEC)} & \text{Male body length } 28 \text{ d: } 440 \\ \text{Female body length } 28 \text{ d: } \\ 843 \\ \hline \text{Time to first brood: } 440 \\ \hline \text{\% control at NOEC} & G_1 \text{ survival: } \\ \hline 7 \text{ d: } 95 \\ \hline 14 \text{ d: } 95 \\ \hline 21 \text{ d: } 102 \\ \hline \end{array} & \begin{array}{c} G_1 \text{ survival: } \\ \hline 7 \text{ d: } 93 \text{ (tmt) } / 98 \\ \hline \text{(control) = } 95 \\ \hline \end{array} $		e body length 28 d:	
$\begin{array}{c c} \text{MATC (GeoMean NOEC, LOEC)} & \text{Male body length 28 d: } \\ & \text{Female body length 28 d: } \\ & 843 \\ & \text{Time to first brood: } 440 \\ \\ \% & \text{control at NOEC} & \begin{array}{c} G_1 \text{ survival: } \\ 7 \text{ d: } 95 \\ 14 \text{ d: } 95 \\ 21 \text{ d: } 102 \end{array} & \begin{array}{c} G_1 \text{ survival: } \\ 7 \text{ d: } 93 \text{ (tmt) } / 98 \\ \text{(control) = } 95 \end{array} \end{array}$			
	ATC (GeoMean NOEC, LOEC)		
		e body length 28 d:	
% control at NOEC G_1 survival: G_1 survival: 7 d: 95 14 d: 95 14 d: 95 14 d: 102 G_2 survival: G_3 survival: G_4 survival: G_5 control) = 95			
7 d: 95 14 d: 95 21 d: 102 7 d: 93 (tmt) / 98 (control) = 95			
14 d: 95 21 d: 102 (control) = 95	control at NOEC		_
21 d: 102			, , ,
			(control) = 95
			14 1 02 (4 4) / 00
28 d: 91) 1	` /
(control) = 95		1 ' 1	(control) = 95
G ₂ 10 d survival: 4 d: 100 21 d: 100 (tmt) / 98			21 d. 100 (tmt) / 00
			21 d: 100 (tmt) / 98
10 d: 100 (control) = 102		100	(control) = 102
Male body length 14 d: 98 28 d: 89 (tmt) / 98		oody length 14 d: 98	28 d: 89 (tmt) / 98
Male body length 28 d: 99 (control) = 91		• •	, , ,
Female body length 28 d: 96		•	(**************************************
Time to first brood: 102 G_2 10 d survival:		•	G ₂ 10 d survival:
1170			1170
4 d: 100 (tmt) / 100			4 d: 100 (tmt) / 100
(control) = 100			(control) = 100
10 d: 100 (tmt) /			` ,
100 (control) = 100			100 (control) = 100
Male body length			
$\frac{14 \text{ d}}{5.14}$ (max) $\sqrt{5.22}$			
5.14 (tmt) / 5.23			, ,
(control) = 98			, ,
Male body length			1
$\frac{28 \text{ d}}{6.14 \text{ (tmt)} / 6.21}$			
6.14 (lift) / 6.21 (control) = 99			, ,
(control) = 33			
Female body length			Female body length
28 d:			1
$\frac{1}{6.22}$ (tmt) / 6.45			
(control) = 96			, ,

	Lehman 2010	A. bahia
Parameter	Value	Comment
		Time 4 - Cine4 1 1
		Time to first brood:
		16.8 (tmt) / 16.4
		(control) = 102
% control at LOEC	G ₁ survival: not calculable	Male body length
	G ₂ 10 d survival: not	<u>28 d</u> : 608
	calculable	6.05 (tmt) / 6.21
	Male body length 14 d: not	(control) = 97
	calculable	
	Male body length 28 d: 97	Female body length
	Female body length 28 d: 93	<u>28 d</u> : 1170
	Time to first brood: 105	5.97 (tmt) / 6.45
		(control) = 93
		Time to first brood:
		17.3 (tmt) / 16.4
		(control) = 105

Simazine solubility (S) = 5,450 μ g/L, 2S = 10, 900 μ g/L. All exposure test concentrations were acceptable.

Reliability points taken off for:

<u>Documentation:</u> Hardness (2), Alkalinity (2), Conductivity (2), Minimum significant difference (2). Total: 100-6 = 94

<u>Acceptability:</u> Measured concentrations within 20% nominal (4), Hardness (2), Alkalinity (2), Conductivity (1), Minimum significant difference (1). Total: 100-10 =90

Reliability score: mean(92, 90)=91

Anabena flos-aquae

Study: Ma, J., Tong, S., Wang, P. and Chen, J., 2010. Toxicity of Seven Herbicides to the Three Cyanobacteria *Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa*. International Journal of Environmental Research, 4(2), 347-352.

RelevanceReliabilityScore: 82.5Score: 71.5Rating: LRating: L

Relevance points taken off for: Standard method (10), Control response (7.5). 100-17.5 = 82.5

	Ma et al. 2010	Anabena flos- aquae
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Cyanobacteria	
Order	Nostocales	
Family	Nostocaceae	
Genus	Anabena	
Species	Flos-aquae (Lyng.) Breb.	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Algal cells	
Source of organisms	Wuhan Institute of Hydrobiology, the Chinese Academy of Science	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	Given organism size and presence in growth medium, it is assumed that aliquots are inherently randomly
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	24 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/5000 lux	
Dilution water	Growth medium	HGZ medium

	Ma et al. 2010	Anabena flos- aquae
Parameter	Value	Comment
Purity of test substance	98 %	
Concentrations measured?	Not reported	
Toxicity values calculated based on	Nominal	
nominal or measured		
concentrations?		
Concentration of carrier (if any) in	Not reported	
test solutions		
Concentration 1 Nom; Meas (µg/L)	Concentrations not reported	3 reps
Control	Negative	
EC ₅₀ (95% CI) (μg/L)	71.8	Method: linear
		regression

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires distilled water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation:</u> Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-14=86

Acceptability: Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Carrier solvent (4), Adequate organisms per rep (2), Temperature variation (3), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100-43 = 57

Reliability score: mean(86, 57)=71.5

Cyprinus carpio

Study: Velisek, J., Stara, A., Machova, J., Dvorak, P., Zuskova, E. and Svobodova, Z., 2012. Effects of low-concentrations of simazine on early life stages of common carp (*Cyprinus carpio* L.). Neuro. Endocrinol. Lett, 33, 90-95.

RelevanceReliabilityScore: 77.5Score: 77.5Rating: LRating: R

Relevance points taken off for: Endpoint (15), Control described (7.5). 100-22.5=77.5

	Velisek et al. 2012	C. carpio
Parameter	Value	Comment
Test method cited	OECD 210, Fish, Early-life	
	stage toxicity test	
Phylum/subphylum	Chordata	
Class	Actinopterygii	
Order	Cypriniformes	
Family	Cyprinidae	
Genus	Cyprinus	
Species	carpio	
Family native to North America?	Introduced	
Age/size at start of test/growth	Eggs, 24 h post-fertilization	
phase		
Source of organisms	Research Institute of Fish	
	Culture and Hydrobiology	
	in Vodnany, University of	
	South Bohemia, Czech	
	Republic	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Yes	
Test duration	36 d	
Data for multiple times?	8, 19, 25, 32, 36 d	
Effect 1	Mortality	
Control response 1	100% survival	
Effect 2	Growth	
Control response 2	8 d: 1.84 mg	
	36 d: 94.95 mg	
Temperature	20.2 ± 0.9 °C	
Test type	Static renewal	Twice daily
Photoperiod/light intensity	16:8 h light:dark/not	

	Velisek et al. 2012	C. carpio
Parameter	Value	Comment
	reported	
Dilution water	Aerated tap water	
pН	7.2-8.1	
Hardness	Not reported	
Alkalinity	Not reported	
Conductivity	Not reported	
Dissolved Oxygen	>93 %	
Feeding	Live brine shrimp (Artemia	
	salina), ad libitum	
Purity of test substance	99.5 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	≥93 %	
Toxicity values calculated based on	Nominal	
nominal or measured		
concentrations?		
Chemical method documented?	HPLC	
Concentration of carrier (if any) in	None used.	
test solutions		
Concentration 1 Nom; Meas (µg/L)	0.06 μg/L;	reps, 100/rep
Concentration 2 Nom; Meas (µg/L)	60 μg/L;	
Concentration 3 Nom; Meas (µg/L)	600 μg/L;	
Concentration 4 Nom; Meas (µg/L)	3000 μg/L;	
Control	Negative: 0;	
NOEC	0.06 μg/L	Method: probit
	Based on histopathology	p: < 0.05
		MSD: not reported
LOEC	60 µg/L	
	Based on histopathology	
MATC (GeoMean NOEC, LOEC)	1.9	
% control at NOEC	Not calculable	
% control at LOEC	Not calculable	

Notes: LOEC/NOEC based on histopathological changes of cranial kidney, not on growth or mortality.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Control type (8), Measured concentrations (3), Hardness (2), Alkalinity (2), Conductivity (2), Statistical significance (2), Minimum significant difference (2). Total: 100-21=79

<u>Acceptability:</u> Appropriate control (6), Hardness (2), Alkalinity (2), Conductivity (1), Number of concentrations (3), Adequate replication (2), Dilution factor (2), Hypothesis tests (3), Point estimates (3). Total: 100-24 = 76

Reliability score: mean(79,76)=77.5

Chlorella pyrenoidosa

Study: Ma, J., 2002. Differential sensitivity to 30 herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Bulletin of environmental contamination and toxicology, 68(2), 275-281.

RelevanceReliabilityScore: 82.5Score: 75.5Rating: LRating: R

Relevance points taken off for: Acceptable standard (10), Control response (7.5). 100 - 17.5 = 82.5.

	Ma 2002	C. pyrenoidosa
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Chlorophyta	
Class	Trebouxiophyceae	
Order	Chlorellales	
Family	Chlorellaceae	
Genus	Chlorella	
Species	pyrenoidosa	
Family native to North America?	Yes	
Age/size at start of test/growth	Algal cells, initial	
phase	concentration 8 x 10 ⁵ mL ⁻¹	
Source of organisms	Institute of Wuhan	
	Hydrobiology, Chinese	
	Academy of Science	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	25 ± 0 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/5000 lux/cm ⁻²	
Dilution water	HB-4 medium	Li, 1959
Feeding	Growth medium	
Purity of test substance	92.2 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	

	Ma 2002	C. pyrenoidosa
Parameter	Value	Comment
Toxicity values calculated based on	Nominal, although	
nominal or measured	concentrations not reported	
concentrations?		
Chemical method documented?	No	
Concentration of carrier (if any) in	Distilled water, acetone, or	
test solutions	methanol but not specified	
	for simazine	
Concentration Nom; Meas (µg/L)	Range of concentrations: 0-	$3 \text{ reps}, 4x10^5$
	150,000 μg/L, unspecified	cells/rep
Control	Negative	
EC ₅₀ (95% CI) (μg/L)	82	Method: Linear
_		regression

Notes: Growth medium characteristics not reported. Chemical exposure concentrations not reported, only range given for linear regression analysis.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires deionized water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation</u>: Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100 - 14 = 86

Acceptability: Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Carrier solvent (4), Temperature variation (3), Number of concentrations (3), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100 - 35 = 65

Reliability score: mean (86, 65) = 75.5

Cyprinodon variegatus

Study: Murphy, D and Swigert JP. 1992. Simazine: a 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*). Wildlife International Limited, Easton, Maryland. Laboratory project number 108A-143. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503702. CA DPR 138083.

RelevanceReliabilityScore: 85Score: 79Rating: LRating: R

Relevance points taken off for: Freshwater (15). 100-15=85

	Murphey & Swigert 1992	C. variegatus
Parameter	Value	Comment
Test method cited	Pesicide Assessment	
	Guidelines, Sudivision E	
	Hazard Evaluation: Wildlife	
	and Aquatic Organisms and	
	ASTM Standard E	
Phylum/subphylum	Chordata	
Class	Actinopterygii	
Order	Cyprinodontiformes	
Family	Cyprinodontidae	
Genus	Cyprinodon	
Species	variegatus	
Family native to North America?	Yes	
Age/size at start of test/growth	Juveniles: 0.36 g, 22 mm	
phase		
Source of organisms	Laboratory cultures	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	50 h;Yes	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Yes	
Test duration	96 h	
Data for multiple times?	4, 24, 48, 72, 96 h	
Effect 1	Mortality	
Control response 1	0 %	
Temperature	22 ± 1 °C	
Test type	Flow-through	
Photoperiod/light intensity	16 l: 8 d; 344 lux	
Dilution water	Natural seawater, filtered,	Indian River Inlet,
	and diluted with well water	Delaware
		Salinity: 25 ‰

	Murphey & Swigert 1992	C. variegatus
Parameter	Value	Comment
рН	8.3	
Hardness	Not reported	
Alkalinity	Not reported	
Conductivity	Not reported	
Dissolved Oxygen	Not reported	
Feeding	During holding only	Flaked fish food, salmon mash, and/or salmon starter (Zeigler Brothers, Gardners, Pennsylvania); live brine shrimp (Artemia, Newark California); frozen brine shrimp nauplii (Kordon, Hayward, California).
Purity of test substance	Technical grade	Label: 96.9 %
Concentrations measured?	Yes	
Measured is what % of nominal?	62-123 %	
Toxicity values calculated based on nominal or measured concentrations?	Measured	
Chemical method documented?	GC	
Concentration of carrier (if any) in test solutions	Dimethyl formamide, 1200 µg/L	
Concentration 1 Nom; Meas (µg/L)	800; 860	2 reps, 10/rep
Concentration 2 Nom; Meas (µg/L)	1300; 1500	- r - , - · r
Concentration 3 Nom; Meas (µg/L)	2200; 2600	
Concentration 4 Nom; Meas (µg/L)	3600; 4300	
Concentration 4 Norm; Meas (µg/L) Concentration 5 Norm; Meas (µg/L)	6000; 4300	
Control	Negative: 0; 0 Solvent: 0; 0	
LC ₅₀ (95% CI) (μg/L)	> 4300 μg/L	Method: not reported
NOEC	4300 μg/L	Method: visual inspection p: n/a MSD: not reported
% control at NOEC	100% survival	

Notes: LC₅₀ could not be calculated based on mortality results.

Simazine solubility (S) = 5,450 $\mu g/L$, 2S = 10, 900 $\mu g/L$. All exposure concentrations were acceptable.

Reliability points taken off for:

<u>Documentation:</u> Hardness (2), Alkalinity (2), Dissolved oxygen (4), Hypothesis tests (8), % control at NOEC/LOEC (2). Total: 100-18 =82

<u>Acceptability:</u> Measured concentrations within 20% nominal (4), Carrier solvent (4), Hardness (2), Alkalinity (2), Dissolved oxygen (6), Conductivity (1), Minimum significant difference (1), % control at NOEC (1), Point estimates (3). Total: 100- 24=76

Reliability score: mean(82,76)=79

Chlorella vulgaris

Study: Ma, J., Xu, L., Wang, S., Zheng, R., Jin, S., Huang, S., & Huang, Y. 2002. Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. Ecotoxicology and environmental safety, 51(2), 128-132.

RelevanceReliabilityScore: 90Score: 61.5Rating: RRating: L

Relevance points taken off for: Acceptable standard (10). 100 - 10 = 90.

	Ma et al. 2002	C. vulgaris
Parameter	Value	Comment
Test method cited	Not reported	
Division	Chlorophyta	
Class	Trebouxiophyceae	
Order	Chlorellales	
Family	Chlorellaceae	
Genus	Chlorella	
Species	vulgaris	
Family native to North America?	Yes	
Age/size at start of test/growth	Algal cells, initial	
phase	concentration 8 x 10 ⁵ mL ⁻¹	
Source of organisms	Institute of Wuhan	
	Hydrobiology, Chinese	
	Academy of Science	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	25 ± 0 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/500 lx/cm ⁻²	
Dilution water	HB-4 medium	Li, 1959
Feeding	Growth medium	
Purity of test substance	92.2 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Nominal, although	

	Ma et al. 2002	C. vulgaris
Parameter	Value	Comment
nominal or measured concentrations?	concentrations not reported	
Chemical method documented?	No	
Concentration of carrier (if any) in test solutions	Distilled water, acetone, or methanol but not specified for simazine	
Concentration Nom; Meas (µg/L)	Range of concentrations: 0-150, unspecified	3 reps, /rep
Control	Negative	
EC ₅₀ (95% CI) (μg/L)	2173.8	Method: Linear regression of transformed concentration as In data versus % inhibition p: 0.0031

Notes: Growth medium characteristics not reported. Chemical exposure concentrations not reported, only range given for linear regression analysis.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Nominal concentrations (3), Measured concentrations (3), Hardness (2), Alkalinity (2), Dissolved oxygen (4), Conductivity (2), pH (3), Statistical significance (2), Significance level (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100 - 32 = 73

Acceptability: Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Carrier solvent (4), Dilution water (2), Hardness (2), Alkalinity (2), Dissolved oxygen (6), Temperature range (3), Conductivity (1), pH (2), Number of concentrations (3), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100 - 50 = 50

Reliability score: mean (73, 50) = 61.5

Daphnia magna

Study: Marchini, S., Passerini, L., Cesareo, D. and Tosato, M.L., 1988. Herbicidal triazines: Acute toxicity on Daphnia, fish, and plants and analysis of its relationships with structural factors. Ecotoxicology and environmental safety, 16(2), 148-157.

RelevanceReliabilityScore: 85Score: 65.5Rating: LRating: L

Relevance points taken off for: Controls (15). 100-15=85

	Marchini et al. 1988	D. magna
Parameter	Value	Comment
Test method cited	OECD Guidelines No. 202	
Phylum/subphylum	Arthropoda/Crustacea	
Class	Branchiopoda	
Order	Cladocera	
Family	Daphniidae	
Genus	Daphnia	
Species	magna	
Family native to North America?	Yes	
Age/size at start of test/growth	< 24 h	
phase		
Source of organisms	Laboratory cultures	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	48 h	
Data for multiple times?	24, 48 h	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	$21 \pm 1 {}^{\circ}\text{C}$	
Test type	Static	
Photoperiod/light intensity	12:12/not reported	
Dilution water	Dechlorinated, oxygen	
	saturated tap water	
рН	8.4	
Hardness	250 mg/L CaCO ₃	
Alkalinity	Not reported but stated	
	within reported limits	
Conductivity	Not reported but stated	
	within reported limits	

	Marchini et al. 1988	D. magna
Parameter	Value	Comment
Dissolved Oxygen	Not reported	
Feeding	Chlorella, daily	
Purity of test substance	96-99 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	UV-vis	
Concentration of carrier (if any) in	Not used	
test solutions		
Concentration 1 Nom; Meas (µg/L)	Concentrations not reported	4 reps, 20/rep
Control	Not reported	
EC ₅₀ (95% CI) (μg/L)	24 h: > 3500	Method: Litchefield
	48 h: > 3500	and Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Control type (8), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Hypothesis tests (8). Total: 100- 26=74

Acceptability: Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Feeding (3), Dissolved oxygen (6), Conductivity (1), pH (2), Number of concentrations (3), Random design (2), Dilution factor (2), Hypothesis tests (3). Total: 100- 43=57

Reliability score: mean(74, 57)=65.5

Danio rerio

Study: Plhalova, L., Haluzova, I., Macova, S., Dolezelova, P., Praskova, E., Marsalek, P., Skoric, M., Svobodova, Z., Pistekova, V. and Bedanova, I., 2010. Effects of subchronic exposure to simazine on zebrafish (*Danio rerio*). Neuro endocrinology letters, 32, 89-94.

RelevanceReliabilityScore: 85Score: 68.5Rating: LRating: L

Relevance points taken off for: Endpoint (15). 100-15 = 85

	Plhalova et al. 2010	D. rerio
Parameter	Value	Comment
Test method cited	OECD method number 215,	
	Juvenile Growth Test	
Phylum/subphylum	Chordata	
Class	Actinopterygii	
Order	Cypriniformes	
Family	Cyprinidae	
Genus	Danio	
Species	rerio	
Family native to North America?	Introduced	
Age/size at start of test/growth	20 d	
phase		
Source of organisms	Not reported	
Have organisms been exposed to	Not reported	
contaminants?		
Animals acclimated and disease-	Not reported	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Not reported	
Test duration	28 d	
Data for multiple times?	No data reported	
Effect 1	Mortality	
Control response 1	≤ 5%	
Effect 2	Histopathology	
Control response 2	Not reported	
Temperature	23 ± 2 °C	
Test type	Flow-through	
Photoperiod/light intensity	Not reported	
Dilution water	Not reported	
рН	7.98-8.33	
Hardness	Not reported	
Alkalinity	Not reported	
Conductivity	Not reported	

	Plhalova et al. 2010	D. rerio
Parameter	Value	Comment
Dissolved Oxygen	>5.1 mg/L	> 60%
Feeding	Dried Artemia salina	8% of fish body weight/d
Purity of test substance	99.5 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	81-93 %	
Toxicity values calculated based on nominal or measured concentrations?	Nominal	
Chemical method documented?	GC/IT-MS	
Concentration of carrier (if any) in test solutions	Dimethyl sulfoxide 0.1 mL/L	
Concentration 1 Nom; Meas (µg/L)	0.06; not reported	2 reps, 40/rep
Concentration 2 Nom; Meas (µg/L)	0.6; not reported	2 reps, 40/rep
Concentration 3 Nom; Meas (µg/L)	6.0; not reported	2 reps, 40/rep
Concentration 4 Nom; Meas (µg/L)	60.0; not reported	2 reps, 40/rep
Control	Negative: 0; not reported Solvent: 0; not reported	2 reps, 40/rep
NOEC	6.0 µg/L, based on histopathology, not mortality	Method: ANOVA, Dunnett's test p: 0.05 MSD: not reported
LOEC	60.0 µg/L, based on histopathology, not mortality	
MATC (GeoMean NOEC, LOEC)	19	
% control at NOEC	Not calculable	
% control at LOEC	Not calculable	

Notes: Raw data not included. NOEC/LOEC based on histopathology, not mortality.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Organism source (5), Measured concentrations (3), Dilution water (3), Hardness (2), Alkalinity (2), Conductivity (2), Photoperiod (3), Minimum significant difference (2), % control at NOEC/LOEC (2), Point estimates (8). Total: 100- 32=64

<u>Acceptability:</u> No prior contamination (4), Acclimation (1), Dilution water (2), Hardness (2), Alkalinity (2), Conductivity (1), Temperature variation (3), Photoperiod (2), Random design (2), Dilution factor (2), Hypothesis tests (3), Point estimates (3). Total: 100-27 = 73

Reliability score: mean(64,73)=68.5

Microcystis aerunginosa

Study: Ma, J., Tong, S., Wang, P. and Chen, J., 2010. Toxicity of Seven Herbicides to the Three Cyanobacteria *Anabaena flos-aquae*, *Microcystis flos-aquae* and *Microcystis aeruginosa*. International Journal of Environmental Research, 4(2), 347-352.

RelevanceReliabilityScore: 82.5Score: 71.5Rating: LRating: L

Relevance points taken off for: Standard method (10), Control response (7.5). 100-17.5 = 82.5

	Ma et al. 2010	M. aerunginosa
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Cyanophyceae	
Order	Chroococcales	
Family	Microcystaceae	
Genus	Microcystis	
Species	aeruginosa	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Algal cells	
Source of organisms	Wuhan Institute of	
	Hydrobiology, the Chinese	
	Academy of Science	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	Given organism size and presence in growth medium, it is assumed that aliquots are inherently randomly
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	24 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/5000 lux	
Dilution water	Growth medium	HGZ medium
Purity of test substance	98 %	

	Ma et al. 2010	M. aerunginosa
Parameter	Value	Comment
Concentrations measured?	Not reported	
Toxicity values calculated based on	Nominal	
nominal or measured		
concentrations?		
Concentration of carrier (if any) in	Not reported	
test solutions		
Concentration 1 Nom; Meas (µg/L)	Concentrations not reported	3 reps
Control	Negative	
EC ₅₀ (95% CI) (μg/L)	304	Method: linear
		regression

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires distilled water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation:</u> Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-14=86

Acceptability: Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Carrier solvent (4), Adequate organisms per rep (2), Temperature variation (3), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100-43 = 57

Reliability score: mean(86, 57)=71.5

Microcystis flos-aquae

Study: Ma, J., Tong, S., Wang, P. and Chen, J., 2010. Toxicity of Seven Herbicides to the Three Cyanobacteria *Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa*. International Journal of Environmental Research, 4(2), 347-352.

RelevanceReliabilityScore: 82.5Score: 71.5Rating: LRating: L

Relevance points taken off for: Standard method (10), Control response (7.5). 100-17.5 = 82.5

	Ma et al. 2010	Microcystis flos- aquae
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Cyanophyceae	
Order	Chroococcales	
Family	Microcystaceae	
Genus	Microcystis	
Species	flos-aquae	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Algal cells	
Source of organisms	Wuhan Institute of Hydrobiology, the Chinese Academy of Science	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	Given organism size and presence in growth medium, it is assumed that aliquots are inherently randomly
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	24 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/5000 lux	
Dilution water	Growth medium	HGZ medium

	Ma et al. 2010	Microcystis flos- aquae
Parameter	Value	Comment
Purity of test substance	98 %	
Concentrations measured?	Not reported	
Toxicity values calculated based on nominal or measured concentrations?	Nominal	
Concentration of carrier (if any) in test solutions	Not reported	
Concentration 1 Nom; Meas (µg/L)	Concentrations not reported	3 reps
Control	Negative	
EC ₅₀ (95% CI) (μg/L)	110	Method: linear regression

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires distilled water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation:</u> Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-14=86

<u>Acceptability:</u> Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Carrier solvent (4), Adequate organisms per rep (2), Temperature variation (3), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100-43 =57

Reliability score: mean(86, 57)=71.5

Myriophyllum aquaticum L.

Study: Wilson, P.C., Whitwell, T. and Klaine, S.J., 2001. Simazine toxicity and uptake by parrotfeather. Journal of Aquatic Plant Management, 39, 112-116.

RelevanceReliabilityScore: 82.5Score: 73Rating: LRating: L

Relevance points taken off for: Standard method (10), Control response (7.5). 100-17.5=82.5

	Wilson et al. 2001	M. aquaticum L.
Parameter	Value	Comment
Test method cited	Not reported	
Order	Saxifragales	
Family	Haloragaceae	
Genus	Myriophyllum	
Species	agauticum	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Not reported	
Source of organisms	Carolina Biological Supply,	
	Burlington, North Carolina	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	
Test vessels randomized?	Yes	
Test duration	7 d exposure	
	7 d recovery	
Data for multiple times?	No	
Effect 1	Growth	Fresh weight gain
Control response 1	Not reported	
Temperature	25 ± 2 °C	
Test type	Static	
Photoperiod/light intensity	16:8 light:dark; 375	
	μmol/m ⁻² /s	
Dilution water	10% Hoagland's nutrient	
	medium	
рН	Not reported	
Dissolved Oxygen	Not reported	Not aerated during test
Feeding	Nutrient medium	
Purity of test substance	99.6 %	
Concentrations measured?	Not reported	

	Wilson et al. 2001	M. aquaticum L.
Parameter	Value	Comment
Toxicity values calculated based on	Nominal	
nominal or measured		
concentrations?		
Concentration of carrier (if any) in	Not used	
test solutions		
Concentration 1 Nom; Meas (µg/L)	10; not reported	Reps not reported
Concentration 2 Nom; Meas (µg/L)	30; not reported	
Concentration 3 Nom; Meas (µg/L)	100; not reported	
Concentration 4 Nom; Meas (µg/L)	300; not reported	
Concentration 5 Nom; Meas (µg/L)	1000; not reported	
Concentration 6 Nom; Meas (µg/L)	3000; not reported	
Control	0; 0	
NOEC	Growth: 100 µg/L	Method:
		ANOVA/LSD
		p: 0.05
		MSD: not reprorted
LOEC	Growth: 300 µg/L	
MATC (GeoMean NOEC, LOEC)	173	
% control at NOEC	Not calculable	
% control at LOEC	Not calculable	

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Water quality parameters not reported and points were not deducted because this is a standard nutrient medium.

Reliability points taken off for:

<u>Documentation</u>: Organism life stage/size (5), Measured concentrations (3), Minimum significant difference (2), % control at NOEC/LOEC (2), Point estimates (8). Total: 100-20 =80

<u>Acceptability:</u> Standard method (5), Control response (9), Measured concentrations (4), Organism size (3), Adequate organisms per rep (2), Temperature variation (3), Adequate replication (2), Hypothesis tests (3), Point estimates (3). Total: 100-34 =66

Reliability score: mean(80, 66)=73

Raphidocelis subcapitata

Study: Ma, J., Wang, S., Ma, L., Chen, X., Xu, R. 2006. Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*. Ecotoxicology and Environmental Safety. 63, 456-462.

RelevanceReliabilityScore: 82.5Score: 77Rating: LRating: R

Relevance points taken off for: Standard method (10), Control response (7.5). 100 - 17.5 = 82.5

	Ma et al. 2006	R. subcapitata
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Chlorophyta	
Class	Chlorophyceae	
Order	Sphaeropleales	
Family	Selenastraceae	
Genus	Raphidocelis	
Species	subcapitata	
Family native to North America?	Yes	
Age/size at start of test/growth	Exponential	
phase	-	
Source of organisms	Institute of Wuhan	
	Hydrobiology, Chinese	
	Academy of Science	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Yes	15 mL aliquots
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	25 °C	Range not reported
Test type	Static	
Photoperiod/light intensity	450 E m ⁻² s ⁻¹	
Dilution water	Growth medium	Prepared with
		distilled water;
		Chinese National
		Environmental
		Protection Agency
		Guidelines 201,
		HB-4 medium

	Ma et al. 2006	R. subcapitata
Parameter	Value	Comment
pH	Not reported	
Hardness	Not reported	
Alkalinity	Not reported	
Conductivity	Not reported	
Dissolved Oxygen	Not reported	
Feeding	Growth medium	
Purity of test substance	92 %	Technical product
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	Acetone (< 0.05% in	
test solutions	medium) or distilled water	
Concentrations Nom; Meas (µg/L)	Not reported; "A wide range	$3 \text{ reps}, 5 \times 10^4$
	of concentrations" was	cells/rep
	tested	
Control	0, not reported	
EC ₅₀ (95% CI) (μg/L)	748.5	Method: Linear
		regression analysis
		of transformed
		herbicide
		concentration as
		natural log data vs.
		% inhibition

Simazine solubility (S) = 5,450 ug/L, 2S = 10,900 µg/L.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires distilled water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation</u>: Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100 - 14 = 86

Acceptability: Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Temperature range (3), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100 - 52 = 68

Reliability score: mean (86, 68) = 77

Skeletonema costatum

Study: Thompson, SG. (1992) A five-day toxicity test with the marine diatom (*Skeletonema costatum*). Wildlife International Limited, Easton, Maryland. Laboratory study number 108A-140. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 42503705. CA DPR 138088.

RelevanceReliabilityScore: 85Score: 95.5Rating: LRating: R

Relevance points taken off for: Freshwater (15). 100-15=85

	Thompson 1992	S. costatum
Parameter	Value	Comment
Test method cited	Pesticide Assessment	
	Guidelines, Subdivision J,	
	Hazard Evaluation: Non-	
	target Plants; Bioassay	
	Procedures for the Ocean	
	Disposal Permit Program;	
	40CFR: Freshwater and	
	Marine Algae Acute	
	Toxicity Test; ASTM,	
	Standard Guide for	
	Conducting static 96-hour	
	Toxicity tests with	
	Micralgae	
Phylum/subphylum	Bacillariophyta	
Class	Coscinodiscophyceae/	
	Thalassiosirophycidae	
Order	Thalassiosirales	
Family	Skeletonemaceae	
Genus	Skeletonema	
Species	costatum	
Family native to North America?	Yes	
Age/size at start of test/growth	NR	
phase		
Source of organisms	Laboratory cultures	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	Given organism
		size and presence in
		growth medium, it

	Thompson 1992	S. costatum
Parameter	Value	Comment
		is assumed that
		aliquots are
		inherently randomly
Test vessels randomized?	Not reported	
Test duration	5 d	
Data for multiple times?	No	
Effect 1	Growth rate	
Control response 1	Negative: 0.4548	
	Solvent: 0.4609	
Temperature	20 ± 2 °C	
Test type	Static	
Photoperiod/light intensity	16:8/4306 lux	
Dilution water	Saltwater algal medium	Saltwater salinity 30 ppt
рН	7.7-8.0	
Feeding	Growth medium	
Purity of test substance	96.5 %	
Concentrations measured?	Yes	
Measured is what % of nominal?	100-110%	
Toxicity values calculated based on	Measured	
nominal or measured		
concentrations?		
Chemical method documented?	GC	
Concentration of carrier (if any) in	Dimethyl formamide 0.4	
test solutions	mL/L	
Concentration 1 Nom; Meas (µg/L)	125; 130	3 reps, 1.0×10^4 cells/rep
Concentration 2 Nom; Meas (µg/L)	250; 250	
Concentration 3 Nom; Meas (µg/L)	500; 520	
Concentration 4 Nom; Meas (µg/L)	1000; 1000	
Concentration 5 Nom; Meas (µg/L)	2000; 2100	
Control	Negative: 0; 0	
	Solvent: 0; 0	
EC ₅₀ (95% CI) (μg/L)	1040 (1000-2100)	Method: binomial
NOEC	250	Method: binomial
		p: not reported
		MSD: not reported
% control at NOEC	Growth rate: 101 %	0.4611 (tmt)/
		0.4579 (mean
		controls) = 101

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for

algal/plant studies, the growth medium used requires distilled water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation:</u> Significance level (2), Minimum significant difference (2). Total: 100-4 = 96

<u>Acceptability:</u> Temperature variation (3), Minimum significant difference (1), % control at LOEC (1). Total: 100-5 =95

Reliability score: mean(95,96)=95.5

Scenedesmus obliquus

Study: Ma, J., 2002. Differential sensitivity to 30 herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Bulletin of environmental contamination and toxicology, 68(2), 275-281.

RelevanceReliabilityScore: 82.5Score: 75.5Rating: LRating: R

Relevance points taken off for: Acceptable standard method (10), Control response (7.5). 100 - 17.5 = 82.5.

	Ma 2002	S. obliquus
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Chlorophyta	
Class	Chlorophyceae	
Order	Sphaeropleales	
Family	Scenedesmaceae	
Genus	Scenedesmus	
Species	obliquus	
Family native to North America?	Yes	
Age/size at start of test/growth	Algal cells, initial	
phase	concentration 8 x 10 ⁵ mL ⁻¹	
Source of organisms	Institute of Wuhan	
	Hydrobiology, Chinese	
	Academy of Science	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	25 ± 0 °C	
Test type	Static	
Photoperiod/light intensity	Continuous/5000 lux/cm ⁻²	
Dilution water	HB-4 medium	Li, 1959
Feeding	Growth medium	
Purity of test substance	92.2 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	

	Ma 2002	S. obliquus
Parameter	Value	Comment
Toxicity values calculated based on	Nominal, although	
nominal or measured	concentrations not reported	
concentrations?		
Chemical method documented?	No	
Concentration of carrier (if any) in	Distilled water, acetone, or	
test solutions	methanol but not specified	
	for simazine	
Concentration Nom; Meas (µg/L)	Range of concentrations: 0-	$3 \text{ reps}, 4x10^5$
	150,000 μg/L, unspecified	cells/rep
Control	Negative	
EC ₅₀ (95% CI) (μg/L)	257	Method: Linear
		regression

Notes: Growth medium characteristics not reported. Chemical exposure concentrations not reported, only range given for linear regression analysis.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points were not taken off for water quality parameters (hardness, alkalinity, conductivity) because there is no guidance for these parameters in the test guidelines for algal/plant studies, the growth medium used requires deionized water, and the medium is presumably appropriate for the test species because a specific culture media was used.

Reliability points taken off for:

<u>Documentation</u>: Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100 - 14 = 86

Acceptability: Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Carrier solvent (4), Temperature variation (3), Number of concentrations (3), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100 - 35 = 65

Reliability score: mean (86, 65) = 75.5

Scenedesmus quadricauda

Study: Ma, J., Lin, F., Wang, S. and Xu, L., 2003. Toxicity of 21 herbicides to the green alga *Scenedesmus quadricauda*. *Bulletin of environmental contamination and toxicology*, 71(3), 0594-0601.

RelevanceReliabilityScore: 82.5Score: 70Rating: LRating: L

Relevance points taken off for: Standard method (10), Control response (7.5). 100-17.5 = 82.5

	Ma et al. 2003	S. quadricauda
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Chlorophyta	
Class	Chlorophyceae	
Order	Sphaeropleales	
Family	Scenedesmaceae	
Genus	Scenedesmus	
Species	quadricauda	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Algal cells	
Source of organisms	Institute of Wuhan Hydrobiology, the Chinese Academy of Science	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	Given size of the cells, inherently randomized
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	3, 6, 12, 24, 36 h	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	4 °C	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Growth medium	HB-4
Purity of test substance	92.2 %	
Concentrations measured?	Not reported	
Toxicity values calculated based on	Nominal	

	Ma et al. 2003	S. quadricauda
Parameter	Value	Comment
nominal or measured		
concentrations?		
Concentration 1 Nom; Meas (µg/L)	Concentrations ranged from	3 reps, /rep
	0-150,000 μg/L with a	
	negative control. Details not	
	reported.	
EC ₅₀ (95% CI) (μg/L)	150 (CI not reported)	Method: linear
		regression

Notes: CI not reported.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Photoperiod (3), Minimum significant difference (2), NOEC/LOEC (2). Total: 100-21 =83

Acceptability: Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Carrier solvent (4), Adequate organisms per rep (2), Temperature variation (3), Photoperiod (2), Number of concentrations (3), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100-43 =57

Reliability score: mean(83,57)=70

Appendix B5 - Mesocosm studies rated L

Simazine

Jenkins, D.G. and Buikema, A.L., 1990. Response of a winter plankton food web to simazine. *Environmental toxicology and chemistry*, 9(6), 693-705.

Paper conclusion: "Persistent levels of simazine may not have a deleterious impact on winter zooplankton communities dominated by noncustracean taxa (rotifers and protozoans). Phytoplankton may be differentially affected, but the relative lack of dependence on autotrophs by winter zooplankton communities may mitigate indirect effects via food webs."

Documentation and acceptability (reliability) evaluation for data derived from aquatic outdoor field and indoor model ecosystems experiments. Include notes next to each parameter. Adapted from ECOTOX 2006; Table from TenBrook et al. 2010.

Parameter ^a	Score ^b	Points
Results published or in signed, dated format Published peer review article	5	5
Exposure duration and sample regime adequately described	6	6
Unimpacted site (Score 7 for artificial systems) No simazine detected in controls or ambient waters	7	7
Adequate range of organisms in system (1° producers, 1°, 2° consumers) Range of bacteria, phytoplankton, and zooplankton described.	6	6
Chemical		
Grade or purity stated Formulation, simazine 41.9 %	6	0
Concentrations measured/estimated and reported 100, 500, 1000	8	8
Analysis method stated HPTLC	2	2
Habitat described (e.g., pond, lake, ditch, artificial, lentic, lotic) 0.31 ha impounded farm pond	6	6
Water quality		
Source identified Pond in Montgomery County, Virginia	2	2
Hardness reported Measured but not reported	1	0
Alkalinity reported Measured but not reported	1	0
Dissolved oxygen reported	2	2
Temperature reported	2	2
Conductivity reported Measured but not reported	1	0
pH reported	1	1
Photoperiod reported Ambient conditions, not reported	1	0
Organic carbon reported Not reported	2	0
Chemical fate reported	3	0

Parameter ^a	Score ^b	Points
Geographic location identified (Score 2 for indoor systems) Pond in Montgomery County, Virginia	2	2
Pesticide application		
Type reported (e.g., spray, dilutor, injection) Not reported	2	0
Frequency reported Static	2	2
Date/season reported (Score 2 for indoor systems) 1-22 December 1984	2	2
Test endpoints		
Species abundance reported Relative densities in tables 2, 3 and figure 1	3	3
Species diversity reported	3	3
Biomass reported	2	0
Ecosystem recovery reported	2	0
Statistics		
Methods identified Dunnett's test	2	2
At least 2 replicates	3	3
At least 2 test concentrations and 1 control 3 conc. & control	3	3
Dose-response relationship observed	2	2
Hypothesis tests		
NOEC determined	4	0
Significance level stated α =0.01 or 0.05	2	2
Minimum significant difference reported	2	0
% of control at NOEC and/or LOEC reported or calculable	2	0
Total Reliability	100	71

LOEC = lowest observed effect concentration, NOEC = no observed effect concentration.

^aCompiled from RIVM 2001, USEPA 1985 and 2003a, ECOTOX 2006, CCME 1995, ANZECC and ARMCANZ 2000, OECD 1995a, and van der Hoeven et al. 1997. bWeighting based on ECOTOX 2006 and on data quality criteria in RIVM 2001 and OECD 1995a.

Simazine

Elodea canadensis

Vervliet-Scheebaum M, Straus A, Tremp H, Hamer M, Maund SJ, Wagner E, and Schulz R. (2010) A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes. *Environmental pollution*, 158(2), 615-623.

Documentation and acceptability (reliability) evaluation for data derived from aquatic outdoor field and indoor model ecosystems experiments. Include notes next to each parameter. Adapted from ECOTOX 2006; Table from TenBrook et al. 2010.

Parameter ^a	Score ^b	Points
Results published or in signed, dated format Published peer review article	5	5
Exposure duration and sample regime adequately described	6	6
Unimpacted site (Score 7 for artificial systems) fiberglass tanks inside concrete ponds	7	7
Adequate range of organisms in system (1° producers, 1°, 2° consumers) Rooted macrophytes (P. amphibia, E. canadensis, M. spicatum, and G. maxima) and naturally occurring alga	6	6
Chemical		
Grade or purity stated formulation, GESATOP 500 FW, 50% active ingredient	6	6
Concentrations measured/estimated and reported Nominal: 50, 500, 5000 μg/L; Measured: 80, 1100, 8500 μg/L	8	8
Analysis method stated LC-MS/MS	2	2
Habitat described (e.g., pond, lake, ditch, artificial, lentic, lotic) fiberglass tanks inside concrete ponds	6	6
Water quality		
Source identified Aged tap water	2	2
Hardness reported	1	0
Alkalinity reported	1	0
Dissolved oxygen reported Plotted, approximately 50-140%	2	2
Temperature reported 15-22°C, ambient	2	2
Conductivity reported Plotted, approximately 350-675 µS/cm	1	1
pH reported Plotted, approximately 7.5-9.75	1	1
Photoperiod reported	1	1
Organic carbon reported	2	0
Chemical fate reported	3	3
Geographic location identified (Score 2 for indoor systems) 51°26′58″N, 0°44′58″W	2	2

Parameter ^a	Score ^b	Points
Pesticide application		
Type reported (e.g., spray, dilutor, injection) stirred into water column	2	2
Frequency reported	2	2
Date/season reported (Score 2 for indoor systems) May-September 2004	2	2
Test endpoints		
Species abundance reported Figure 4	3	3
Species diversity reported *See note at end	3	0
Biomass reported Wet and dry weight of some macrophytes only	2	2
Ecosystem recovery reported	2	2
Statistics		
Methods identified Dunnett's test	2	2
At least 2 replicates 3 replicates		3
At least 2 test concentrations and 1 control 3 concentrations, 1 control	3	3
Dose-response relationship observed	2	2
Hypothesis tests		
NOEC determined Based on nominal concentration, length increase of main shoot 84 d: 50 μ g/L		4
Significance level stated 0.05	2	2
Minimum significant difference reported	2	0
% of control at NOEC and/or LOEC reported or calculable Data in table 2	2	2
Total Reliability	100	91

LOEC = lowest observed effect concentration, NOEC = no observed effect concentration.

^aCompiled from RIVM 2001, USEPA 1985 and 2003a, ECOTOX 2006, CCME 1995, ANZECC and ARMCANZ 2000, OECD 1995a, and van der Hoeven et al. 1997.

^bWeighting based on ECOTOX 2006 and on data quality criteria in RIVM 2001 and OECD 1995a.

^{*}Criteria for algae: "the species had to be present in the sample in all three replicates with at least 40.8 individuals per mL per microcosm or they had to be present in at least two out of three replicates per treatment with 204 individuals per mL. This procedure allowed for a reduction of the number of species evaluated and ensured that only those species that were present in high abundance were considered."

Simazine

Glyceria maxima

Vervliet-Scheebaum M, Straus A, Tremp H, Hamer M, Maund SJ, Wagner E, and Schulz R. (2010) A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes. *Environmental pollution*, 158(2), 615-623.

Documentation and acceptability (reliability) evaluation for data derived from aquatic outdoor field and indoor model ecosystems experiments. Include notes next to each parameter. Adapted from ECOTOX 2006; Table from TenBrook et al. 2010.

Parameter ^a	Score ^b	Points
Results published or in signed, dated format Published peer review article		5
Exposure duration and sample regime adequately described	6	6
Unimpacted site (Score 7 for artificial systems) fiberglass tanks inside concrete ponds	7	7
Adequate range of organisms in system (1° producers, 1°, 2° consumers) Rooted macrophytes (P. amphibia, E. canadensis, M. spicatum, and G. maxima) and naturally occurring alga	6	6
Chemical		
Grade or purity stated formulation, GESATOP 500 FW, 50% active ingredient	6	6
Concentrations measured/estimated and reported Nominal: 50, 500, 5000 μg/L; Measured: 80, 1100, 8500 μg/L	8	8
Analysis method stated LC-MS/MS	2	2
Habitat described (e.g., pond, lake, ditch, artificial, lentic, lotic) fiberglass tanks inside concrete ponds	6	6
Water quality		
Source identified Aged tap water	2	2
Hardness reported		0
Alkalinity reported	1	0
Dissolved oxygen reported Plotted, approximately 50-140%	2	2
Temperature reported 15-22°C, ambient	2	2
Conductivity reported Plotted, approximately 350-675 µS/cm	1	1
pH reported Plotted, approximately 7.5-9.75		1
Photoperiod reported	1	1
Organic carbon reported	2	0
Chemical fate reported	3	3
Geographic location identified (Score 2 for indoor systems) 51°26′58″N, 0°44′58″W	2	2

Parameter ^a	Score ^b	Points
Pesticide application		
Type reported (e.g., spray, dilutor, injection) stirred into water column	2	2
Frequency reported	2	2
Date/season reported (Score 2 for indoor systems) May-September 2004	2	2
Test endpoints		
Species abundance reported Figure 4	3	3
Species diversity reported *See note at end	3	0
Biomass reported Wet and dry weight of some macrophytes only	2	2
Ecosystem recovery reported	2	2
Statistics		
Methods identified Dunnett's test	2	2
At least 2 replicates 3 replicates	3	3
At least 2 test concentrations and 1 control 3 concentrations, 1 control	3	3
Dose-response relationship observed	2	2
Hypothesis tests		
NOEC determined Based on nominal concentration Length increase, number of shoots, biomass, 84 d: 50 µg/L		4
Significance level stated 0.05	2	2
Minimum significant difference reported	2	0
% of control at NOEC and/or LOEC reported or calculable Data in table 2	2	2
Total Reliability	100	91

LOEC = lowest observed effect concentration, NOEC = no observed effect concentration.

^aCompiled from RIVM 2001, USEPA 1985 and 2003a, ECOTOX 2006, CCME 1995, ANZECC and ARMCANZ 2000, OECD 1995a, and van der Hoeven et al. 1997.

^bWeighting based on ECOTOX 2006 and on data quality criteria in RIVM 2001 and OECD 1995a.

^{*}Criteria for algae: "the species had to be present in the sample in all three replicates with at least 40.8 individuals per mL per microcosm or they had to be present in at least two out of three replicates per treatment with 204 individuals per mL. This procedure allowed for a reduction of the number of species evaluated and ensured that only those species that were present in high abundance were considered."

Simazine

Myriophyllum spicatum

Vervliet-Scheebaum M, Straus A, Tremp H, Hamer M, Maund SJ, Wagner E, and Schulz R. (2010) A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes. *Environmental pollution*, 158(2), 615-623.

Documentation and acceptability (reliability) evaluation for data derived from aquatic outdoor field and indoor model ecosystems experiments. Include notes next to each parameter. Adapted from ECOTOX 2006; Table from TenBrook et al. 2010.

Parameter ^a	Scoreb	Points
Results published or in signed, dated format Published peer review article		5
Exposure duration and sample regime adequately described	6	6
Unimpacted site (Score 7 for artificial systems) fiberglass tanks inside concrete ponds	7	7
Adequate range of organisms in system (1° producers, 1°, 2° consumers) Rooted macrophytes (P. amphibia, E. canadensis, M. spicatum, and G. maxima) and naturally occurring alga	6	6
Chemical		
Grade or purity stated formulation, GESATOP 500 FW, 50% active ingredient	6	6
Concentrations measured/estimated and reported Nominal: 50, 500, 5000 µg/L; Measured: 80, 1100, 8500 µg/L	8	8
Analysis method stated LC-MS/MS	2	2
Habitat described (e.g., pond, lake, ditch, artificial, lentic, lotic) fiberglass tanks inside concrete ponds		6
Water quality		
Source identified Aged tap water	2	2
Hardness reported		0
Alkalinity reported	1	0
Dissolved oxygen reported Plotted, approximately 50-140%	2	2
Temperature reported 15-22°C, ambient		2
Conductivity reported Plotted, approximately 350-675 µS/cm	1	1
pH reported Plotted, approximately 7.5-9.75	1	1
Photoperiod reported	1	1
Organic carbon reported	2	0
Chemical fate reported	3	3
Geographic location identified (Score 2 for indoor systems) 51°26'58"N, 0°44'58"W	2	2

Parameter ^a	Score ^b	Points
Pesticide application		
Type reported (e.g., spray, dilutor, injection) stirred into water column	2	2
Frequency reported	2	2
Date/season reported (Score 2 for indoor systems) May-September 2004	2	2
Test endpoints		
Species abundance reported Figure 4	3	3
Species diversity reported *See note at end	3	0
Biomass reported Wet and dry weight of some macrophytes only	2	2
Ecosystem recovery reported	2	2
Statistics		
Methods identified Dunnett's test	2	2
At least 2 replicates 3 replicates	3	3
At least 2 test concentrations and 1 control 3 concentrations, 1 control	3	3
Dose-response relationship observed	2	2
Hypothesis tests		
NOEC determined Based on nominal concentration Length increase of main and side shoots, biomass, 84 d: 500 $\mu g/L$		4
Significance level stated 0.05	2	2
Minimum significant difference reported	2	0
% of control at NOEC and/or LOEC reported or calculable Data in table 2	2	2
Total Reliability	100	91

LOEC = lowest observed effect concentration, NOEC = no observed effect concentration.

^aCompiled from RIVM 2001, USEPA 1985 and 2003a, ECOTOX 2006, CCME 1995, ANZECC and ARMCANZ 2000, OECD 1995a, and van der Hoeven et al. 1997.

^bWeighting based on ECOTOX 2006 and on data quality criteria in RIVM 2001 and OECD 1995a.

^{*}Criteria for algae: "the species had to be present in the sample in all three replicates with at least 40.8 individuals per mL per microcosm or they had to be present in at least two out of three replicates per treatment with 204 individuals per mL. This procedure allowed for a reduction of the number of species evaluated and ensured that only those species that were present in high abundance were considered."

Simazine

Persicaria amphibian

Vervliet-Scheebaum M, Straus A, Tremp H, Hamer M, Maund SJ, Wagner E, and Schulz R. (2010) A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes. *Environmental pollution*, 158(2), 615-623.

Documentation and acceptability (reliability) evaluation for data derived from aquatic outdoor field and indoor model ecosystems experiments. Include notes next to each parameter. Adapted from ECOTOX 2006; Table from TenBrook et al. 2010.

Parameter ^a	Scoreb	Points
Results published or in signed, dated format Published peer review article	5	5
Exposure duration and sample regime adequately described	6	6
Unimpacted site (Score 7 for artificial systems) fiberglass tanks inside concrete ponds	7	7
Adequate range of organisms in system (1° producers, 1°, 2° consumers) Rooted macrophytes (P. amphibia, E. canadensis, M. spicatum, and G. maxima) and naturally occurring alga	6	6
Chemical		
Grade or purity stated formulation, GESATOP 500 FW, 50% active ingredient	6	6
Concentrations measured/estimated and reported Nominal: 50, 500, 5000 µg/L; Measured: 80, 1100, 8500 µg/L	8	8
Analysis method stated LC-MS/MS	2	2
Habitat described (e.g., pond, lake, ditch, artificial, lentic, lotic) fiberglass tanks inside concrete ponds		6
Water quality		
Source identified Aged tap water	2	2
Hardness reported		0
Alkalinity reported	1	0
Dissolved oxygen reported Plotted, approximately 50-140%	2	2
Temperature reported 15-22°C, ambient		2
Conductivity reported Plotted, approximately 350-675 µS/cm	1	1
pH reported Plotted, approximately 7.5-9.75	1	1
Photoperiod reported	1	1
Organic carbon reported	2	0
Chemical fate reported	3	3
Geographic location identified (Score 2 for indoor systems) 51°26'58"N, 0°44'58"W	2	2

Parameter ^a	Score ^b	Points
Pesticide application		
Type reported (e.g., spray, dilutor, injection) stirred into water column	2	2
Frequency reported	2	2
Date/season reported (Score 2 for indoor systems) May-September 2004	2	2
Test endpoints		
Species abundance reported Figure 4	3	3
Species diversity reported *See note at end	3	0
Biomass reported Wet and dry weight of some macrophytes only	2	2
Ecosystem recovery reported	2	2
Statistics		
Methods identified Dunnett's test	2	2
At least 2 replicates 3 replicates	3	3
At least 2 test concentrations and 1 control 3 concentrations, 1 control	3	3
Dose-response relationship observed	2	2
Hypothesis tests		
NOEC determined Based on nominal concentration; length increase, number of shoots 84 d: 50 $\mu g/L$		4
Significance level stated 0.05	2	2
Minimum significant difference reported	2	0
% of control at NOEC and/or LOEC reported or calculable Data in table 2	2	2
Total Reliability	100	91

LOEC = lowest observed effect concentration, NOEC = no observed effect concentration.

^aCompiled from RIVM 2001, USEPA 1985 and 2003a, ECOTOX 2006, CCME 1995, ANZECC and ARMCANZ 2000, OECD 1995a, and van der Hoeven et al. 1997.

^bWeighting based on ECOTOX 2006 and on data quality criteria in RIVM 2001 and OECD 1995a.

^{*}Criteria for algae: "the species had to be present in the sample in all three replicates with at least 40.8 individuals per mL per microcosm or they had to be present in at least two out of three replicates per treatment with 204 individuals per mL. This procedure allowed for a reduction of the number of species evaluated and ensured that only those species that were present in high abundance were considered."

Appendix B6 - Aqueous studies rated N

A. fischeri

Study: Hernando, M.D., De Vettori, S., Bueno, M.M. and Fernández-Alba, A.R., 2007. Toxicity evaluation with Vibrio fischeri test of organic chemicals used in aquaculture. *Chemosphere*, 68(4), 724-730.

EC₅₀ is inexact and is estimated to be very close to 2S so study automatically rates N and cannot be used for criteria derivation.

C. auratus

Study: Woodward Research Corporation. No date. Simazine, acute toxicity in goldfish. EPA MRID 23322.

LC₅₀ exceeds 2S so study automatically rates N and cannot be used for criteria derivation.

Ceriodaphnia dubia

Study: Foster, S., Thomas, M. and Korth, W., 1998. Laboratory-derived acute toxicity of selected pesticides to *Ceriodaphnia dubia*. Australasian Journal of Ecotoxicology, 4(1), 53-59.

EC50 exceeds 2S so study automatically rates N and cannot be used in criteria calculation.

Chlorella pyrenoidosa

Study: J. Ma, W. Liang, L. Xu, S. Wang, Y. Wei,1 J. Lu. 2001. Acute Toxicity of 33 Herbicides to the Green Alga Chlorella pyrenoidosa. Bull. Environ. Contam. Toxicol. 66:536–541.

RelevanceReliabilityScore: 75Score: 38.5Rating: LRating: N

Relevance points taken off for: Acceptable standard (or equivalent) method used (10), Controls-Described (i.e., solvent, dilution water, etc.) (7.5), Controls-Response reported and meets acceptability requirements (7.5). Total: 100-25 = 75.

	Ma et al. 2001	C. pyrenoidosa
Parameter	Value	Comment
Test method cited	None	
Phylum/subphylum	Chlorophyta	
Class	Trebouxiophyceae	
Order	Chlorellales	
Family	Chlorellaceae	
Genus	Chlorella	
Species	pyrenoidosa	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Algal cells, 6 x 10 ⁵ cells/mL	
Source of organisms	Laboratory culture	Institute of Wuhan Hydrobiology, Chinese Academy of Science
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	96 hours	
Data for multiple times?	No	
Effect 1	Growth	
Control response 1	Not reported	
Temperature	25 °C	
Test type	Static	
Photoperiod/light intensity	Continuous @ 5000 lux/cm ²	
Dilution water	Liquid HB-4 medium	
рН	Not reported	
Hardness	Not reported	
Alkalinity	Not reported	

	Ma et al. 2001	C. pyrenoidosa
Parameter	Value	Comment
Conductivity	Not reported	
Dissolved Oxygen	Not reported	
Feeding	Growth medium not	
	renewed	
Purity of test substance	92 %	
Concentrations measured?	No	
Measured is what % of nominal?	Not applicable	
Toxicity values calculated based on	Nominal	
nominal or measured		
concentrations?		
Chemical method documented?	Not applicable	
Concentration of carrier (if any) in	Not reported	
test solutions		
Concentration 1 Nom; Meas (µg/L)	Not reported	reps, /rep
Concentration 2 Nom; Meas (µg/L)	Not reported	
Concentration 3 Nom; Meas (µg/L)	Not reported	
Concentration 4 Nom; Meas (µg/L)	Not reported	
Concentration 5 Nom; Meas (µg/L)	Not reported	
Concentration 6 Nom; Meas (µg/L)	Not reported	
Control	Not described	
EC ₅₀ (95% CI) (μg/L)	82	Method: linear
		regression

Notes: No control data reported. Multiple herbicides tested with various solvents used, although unspecified which was used for which herbicide. Control solvent not reported.

EPA guidance recommends algal species for testing, with *C. pyrenoidosa* not being one of them (alternate).

Simazine solubility (S) = 5,450 μ g/L, 2S = 10, 900 μ g/L.

Reliability points taken off for:

<u>Documentation</u>: Control type (8), Organism life stage/size (5), Analytical method (4), Measured concentrations (3), Dilution water (3), Hardness (2), Alkalinity (2), Dissolved oxygen (4), Conductivity (2), pH (3), Methods identified (5), Statistical significance (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100 - 47 = 53

Acceptability: Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Carrier solvent (4), Appropriate size/age/growth phase (3), Organisms randomized (1), Adequate organisms per rep (2), Acclimation (1), Dilution water (2), Hardness (2), Alkalinity (2), Dissolved oxygen (6), Temperature variation (3), Conductivity (1), pH (2), Photoperiod (2), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Statistical method (2), Hypothesis tests (3), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100 - 76 = 24

Reliability score: mean(53,24) = 38.5

Daphnia pulex

Study: Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates*. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.

RelevanceReliabilityScore: 60Score: 60Rating: NRating: L

Relevance points taken off for: Standard method (10), Endpoint (15), Controls (15). 100-40 = 60

	Johnson & Finley 1980	D. pulex
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Arthropoda/Crustacea	
Class	Branchiopoda	
Order	Cladocera	
Family	Daphniidae	
Genus	Daphnia	
Species	pulex	
Family native to North America?	Yes	
Age/size at start of test/growth	Mature	
phase		
Source of organisms	Federal or State hatchery	
	Invertebrates collected from	
	wild and cultured in	
	laboratory	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	48 h	
Data for multiple times?	Not reported	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	21 ± 1 °C	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Reconstituted deionized	
	water	
pН	7.2-7.5	
Hardness	272 mg/L CaCO ₃	
Alkalinity	30-35 mg/L CaCO ₃	

	Johnson & Finley 1980	D. pulex
Parameter	Value	Comment
Conductivity	Not reported	
Dissolved Oxygen	Not reported	Aerated beforehand
Feeding	Not fed	
Purity of test substance	98.1 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	≤0.5 mL/L acetone	
test solutions		
Concentration 1 Nom; Meas (µg/L)	≥6 concentrations tested	2 reps, 10/rep
Control	Not reported	
EC ₅₀ (95% CI) (μg/L)	3700 (2600-5300)	Method: Litchfield and Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation</u>: Control type (8), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8), Statistical significance (2), Significance level (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-35 =65

<u>Acceptability:</u> Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1). Total: 100-45 = 55

Reliability score: mean(65,55)=60

Cypridopsis vidua

Study: Sanders, HO. 1970. Toxicities of some herbicides to six species of freshwater crustaceans. *Journal of Water Pollution Control Federation*, 42, 1544-1550. EPA MRID 45088221.

RelevanceReliabilityScore: 85Score: 52.5Rating: LRating: N

Relevance points taken off for: Controls (15). 100-15=85.

	Sanders 1970	C. vidua
Parameter	Value	Comment
Test method cited	Previously described in peer review	Comment Sanders HO and Cope OB. 1965. The relative toxicities of several pesticides to two spcies of Cladocerans. Transactions of the American Fisheries Society. 95, 165.
Phylum/subphylum	Anthropoda	50ciety. 75, 105.
Class	Ostracoda	
Order	Podocopida	
Family	Cyprididae	
Genus	Cypridopsis	
Species	vidua	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Not reported	
Source of organisms	Laboratory culture	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	24, 48, 96 h	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	21 ± 0.5 °C	
Test type	Static	

	Sanders 1970	C. vidua
Parameter	Value	Comment
Photoperiod/light intensity	Not reported	
Dilution water	Untreated well water	
pH	7.4	
Hardness	272 mg/L CaCO ₃	
Alkalinity	260 mg/L CaCO ₃	
Dissolved Oxygen	Not reported	Non-aerated water
Feeding	Not reported	
Purity of test substance	Technical	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured	_	
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	1.0 mL/L, methanol	
test solutions		
Concentration 1 Nom; Meas (µg/L)	Concentrations, reps not reported	States that 4 or 5 concentrations and appropriate controls used
Control	Not reported	
EC ₅₀ (95% CI) (μg/L)	3200 (CI not reported)	Method: modified
		Litchfield and
		Wilcoxon

Simazine solubility (S) = 5,450 μ g/L, 2S = 10, 900 μ g/L.

Reliability points taken off for:

<u>Documentation</u>: Organism life stage/size (5), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8). Total: 100-32 =68

Acceptability: Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Adequate organisms per rep (2), Feeding (3), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Statistical method (2), Hypothesis tests (3), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100-67 =37

Reliability score: mean(68,37)=52.5

Daphnia magna

Study: Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates*. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.

RelevanceReliabilityScore: 75Score: 60Rating: LRating: L

Relevance points taken off for: Standard method (10), Controls (15). 100-25 = 75

	Johnson & Finley 1980	D. magna
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Arthropoda/Crustacea	
Class	Branchiopoda	
Order	Cladocera	
Family	Daphniidae	
Genus	Daphnia	
Species	magna	
Family native to North America?	Yes	
Age/size at start of test/growth	First instar	
phase		
Source of organisms	Invertebrates collected from	
	wild and cultured in	
	laboratory	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	48 h	
Data for multiple times?	Not reported	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	$21 \pm 1 {}^{\circ}\text{C}$	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Reconstituted deionized	
	water	
pH	7.2-7.5	
Hardness	272 mg/L CaCO ₃	
Alkalinity	30-35 mg/L CaCO ₃	
Conductivity	Not reported	

	Johnson & Finley 1980	D. magna
Parameter	Value	Comment
Dissolved Oxygen	Not reported	Aerated beforehand
Feeding	Not fed	
Purity of test substance	98.1 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	≤0.5 mL/L acetone	
test solutions		
Concentration 1 Nom; Meas (µg/L)	≥6 concentrations tested but	2 reps, 10/rep
, 0	not reported	
Control	Not reported	
EC ₅₀ (95% CI) (μg/L)	1100 (560-2200)	Method: Litchfield
		and Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation</u>: Control type (8), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8), Statistical significance (2), Significance level (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-35 =65

<u>Acceptability:</u> Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1). Total: 100-45 = 55

Reliability score: mean(65,55)=60

Daphnia magna

Study: Sanders, HO. 1970. Toxicities of some herbicides to six species of freshwater crustaceans. *Journal of Water Pollution Control Federation*, 42, 1544-1550. EPA MRID 45088221.

RelevanceReliabilityScore: 85Score: 52.5Rating: LRating: N

Relevance points taken off for: Controls (15). 100-15=85.

	Sanders 1970	D. magna
Parameter	Value	Comment
Test method cited	Previously described in peer review	Sanders HO and Cope OB. 1965. The relative toxicities of several pesticides to two spcies of Cladocerans. Transactions of the American Fisheries Society. 95, 165.
Phylum/subphylum	Arthropoda/Crustacea	,
Class	Branchiopoda	
Order	Cladocera	
Family	Daphniidae	
Genus	Daphnia	
Species	magna	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Not reported	
Source of organisms	Laboratory culture	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	24, 48, 96 h	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	21 ± 0.5 °C	
Test type	Static	

	Sanders 1970	D. magna
Parameter	Value	Comment
Photoperiod/light intensity	Not reported	
Dilution water	Untreated well water	
pH	7.4	
Hardness	272 mg/L CaCO ₃	
Alkalinity	260 mg/L CaCO ₃	
Dissolved Oxygen	Not reported	Non-aerated water
Feeding	Not reported	
Purity of test substance	Technical	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	1.0 mL/L, methanol	
test solutions		
Concentration 1 Nom; Meas (µg/L)	Concentrations, reps not	States that 4 or 5
	reported	concentrations and
		appropriate controls
		used
Control	Not reported	
EC ₅₀ (95% CI) (μg/L)	1000 (CI not reported)	Method: modified
		Litchfield and
		Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Organism life stage/size (5), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8). Total: 100-32 =68

Acceptability: Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Adequate organisms per rep (2), Feeding (3), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Statistical method (2), Hypothesis tests (3), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1). Total: 100-67 =37

Reliability score: mean(68,37)=52.5

Danio rerio

Study: Plhalova, L., Haluzova, I., Macova, S., Dolezelova, P., Praskova, E., Marsalek, P., Skoric, M., Svobodova, Z., Pistekova, V. and Bedanova, I., 2010. Effects of subchronic exposure to simazine on zebrafish (Danio rerio). Neuro endocrinology letters, 32, 89-94.

RelevanceReliabilityScore: 70Score: 57Rating: LRating: N

Relevance points taken off for: Endpoint (15), Controls (30). 100-30 = 70

	Plhalova et al. 2010	D. rerio
Parameter	Value	Comment
Test method cited	OECD method number 215,	
	Juvenile Growth Test	
Phylum/subphylum	Chordata	
Class	Actinopterygii	
Order	Cypriniformes	
Family	Cyprinidae	
Genus	Danio	
Species	Rerio	
Family native to North America?	Introduced	
Age/size at start of test/growth	20 d	
phase		
Source of organisms	Not reported	
Have organisms been exposed to	Not reported	
contaminants?		
Animals acclimated and disease-	Not reported	
free?		
Animals randomized?	Yes	
Test vessels randomized?	Not reported	
Test duration	28 d	
Data for multiple times?	No data reported	
Effect 1	Mortality	
Control response 1	≤ 5%	
Effect 2	Histopathology	
Control response 2	Not reported	
Temperature	23 ± 2 °C	
Test type	Flow-through	
Photoperiod/light intensity	Not reported	
Dilution water	Not reported	
pH	7.98-8.33	
Hardness	Not reported	
Alkalinity	Not reported	
Conductivity	Not reported	

	Plhalova et al. 2010	D. rerio
Parameter	Value	Comment
Dissolved Oxygen	>5.1 mg/L	> 60%
Feeding	Dried Artemia salina	8% of fish body weight/d
Purity of test substance	99.5 %	Weight a
Concentrations measured?	Yes	
Measured is what % of nominal?	81-93 %	
Toxicity values calculated based on nominal or measured concentrations?	Nominal	
Chemical method documented?	GC/IT-MS	
Concentration of carrier (if any) in	Dimethyl sulfoxide 0.1	
test solutions	mL/L	
Concentration 1 Nom; Meas (µg/L)	0.06; not reported	2 reps, 40/rep
Concentration 2 Nom; Meas (µg/L)	0.6; not reported	2 reps, 40/rep
Concentration 3 Nom; Meas (µg/L)	6.0; not reported	2 reps, 40/rep
Concentration 4 Nom; Meas (µg/L)	60.0; not reported	2 reps, 40/rep
Control	Negative: 0; not reported Solvent: 0; not reported	2 reps, 40/rep
NOEC	6.0 µg/L, based on histopathology, not mortality	Method: ANOVA, Dunnett's test p: 0.05 MSD: not reported
LOEC	60.0 µg/L, based on histopathology, not mortality	
MATC (GeoMean NOEC, LOEC)	19	
% control at NOEC	Not calculable	
% control at LOEC	Not calculable	

Notes: Raw data not included. NOEC/LOEC based on histopathology, not mortality.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Control type (8), Organism source (5), Measured concentrations (3), Dilution water source (3), Hardness (2), Alkalinity (2), Conductivity (2), Photoperiod (3), Statistical significance (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-34=66

Acceptability: Appropriate control (6), Control response (9), Concentrations not > 2x solubility (4), Carrier solvent (4), No prior contamination (4), Acclimation (1), Dilution water (2), Hardness (2), Alkalinity (2), Temperature variation (3), Conductivity (1), Photoperiod (2), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Hypothesis tests (3). Total: 100- 52=48

Reliability score: mean(66,48)=57

Penaeus duoarum

Study: Sleight BH. 1973. Acute toxicity of simazine to pink shrimp (*Penaeus duorarum*) and mud crab (*Neopanope*). Bionomics, Inc., Wareham, Massachusetts. Submitted to Ciba-Geigy Corporation, Ardsley, New York. EPA MRID 23331.

LC₅₀ exceeds 2S so study automatically rates N and cannot be used for criteria derivation.

Graphisurus fasciatus

Study: Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates*. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.

RelevanceReliabilityScore: 60Score: 54.5Rating: NRating: N

Relevance points taken off for: Standard method (10), Controls (15), Point estimates (15). 100-40=60

	Johnson & Finley 1980	G. fasciatus
Parameter	Value	Comment
Test method cited	Not reported	
Phylum	Arthropoda	
Class	Insecta	
Order	Coleoptera	
Family	Cerambycidae	
Genus	Graphisurus	
Species	fasciatus	
Family native to North America?	Yes	
Age/size at start of test/growth	M	
phase		
Source of organisms	Invertebrates collected from	
	wild and cultured in	
	laboratory	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	48 h	
Data for multiple times?	Not reported	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	15 ± 1 °C	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Reconstituted deionized	
	water	
рН	7.2-7.5	
Hardness	40-50 mg/L CaCO ₃	
Alkalinity	30-35 mg/L CaCO ₃	

	Johnson & Finley 1980	G. fasciatus
Parameter	Value	Comment
Conductivity	Not reported	
Dissolved Oxygen	Not reported	Aerated beforehand
Feeding	Not fed	
Purity of test substance	98.1 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	≤0.5 mL/L acetone	
test solutions		
Concentration 1 Nom; Meas (µg/L)	≥6 concentrations tested but	2 reps, 10/rep
	not reported	
Control	Not reported	
EC ₅₀ (95% CI) (μg/L)	>100	Method: Litchfield
		and Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation</u>: Control type (8), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8), Statistical significance (2), Significance level (2), Minimum significant difference (2), % control at NOEC/LOEC (2), Point estimates (8). Total: 100-43 =57

Acceptability: Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1), Point estimates (3). Total: 100-48 =52

Reliability score: mean(57,52)=54.5

Simazine

Goldsborough, L.G. and Robinson, G.G.C., 1983. The effect of two triazine herbicides on the productivity of freshwater marsh periphyton. *Aquatic Toxicology*, 4(2), 95-112.

Documentation and acceptability (reliability) evaluation for data derived from aquatic outdoor field and indoor model ecosystems experiments. Include notes next to each parameter. Adapted from ECOTOX 2006; Table from TenBrook et al. 2010.

Parameter ^a	Scoreb	Points
Results published or in signed, dated format Published peer review article	5	5
Exposure duration and sample regime adequately described	6	0
Unimpacted site (Score 7 for artificial systems) natural system	7	0
Adequate range of organisms in system (1° producers, 1°, 2° consumers) Not reported	6	0
Chemical		
Grade or purity stated unformulated, >98 %	6	6
Concentrations measured/estimated and reported	8	8
Analysis method stated	2	
Habitat described (e.g., pond, lake, ditch, artificial, lentic, lotic) Channel of a delta marsh	6	6
Water quality		
Source identified Natural system	2	2
Hardness reported Not reported	1	0
Alkalinity reported Not reported	1	0
Dissolved oxygen reported Gradient reported	2	2
Temperature reported 14.0-24.7 °C	2	2
Conductivity reported Not reported	1	1
pH reported Not reported	1	0
Photoperiod reported 64-565 μE m ⁻² s ⁻¹	1	1
Organic carbon reported Not reported	2	2
Chemical fate reported Not reported	3	0
Geographic location identified (Score 2 for indoor systems) Marsh near Lake Manitoba, Canada	2	2
Pesticide application		
Type reported (e.g., spray, dilutor, injection) Via gauze bag	2	2
Frequency reported	2	2
Date/season reported (Score 2 for indoor systems)	2	0

Parameter ^a	Score ^b	Points
Test endpoints		
Species abundance reported Photosynthetic activity and chlorophyll a endpoints	3	0
Species diversity reported	3	0
Biomass reported	2	0
Ecosystem recovery reported	2	2
Statistics		
Methods identified	2	2
At least 2 replicates	3	3
At least 2 test concentrations and 1 control 0.1, 1.0, 5.0 mg/L and control	3	3
Dose-response relationship observed	2	2
Hypothesis tests		
NOEC determined	4	0
Significance level stated $\alpha = 0.5$	2	2
Minimum significant difference reported	2	0
% of control at NOEC and/or LOEC reported or calculable	2	0
Total	100	55

LOEC = lowest observed effect concentration, NOEC = no observed effect concentration.
^aCompiled from RIVM 2001, USEPA 1985 and 2003a, ECOTOX 2006, CCME 1995, ANZECC and ARMCANZ 2000, OECD 1995a, and van der Hoeven et al. 1997.

^bWeighting based on ECOTOX 2006 and on data quality criteria in RIVM 2001 and OECD 1995a.

Lepomis macrochirus

Study: Beliles, R.P.; Scott, W.; Knott, W. (1965) Simazine: Acute Toxicity in Sunfish. Woodard Research Corporation, Herndon, Virginia. Submitted to Geigy Chemical Corporation, New York, N.Y. EPA MRID 25438.

RelevanceReliabilityScore:Score:Rating:Rating:

 LC_{50} values exceed 2S so study automatically rates N and cannot be used for criteria derivation.

Lepomis macrochirus

Study: Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates*. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.

LD₅₀ exceeds 2S so study rates N and cannot be used for criteria derivation.

Lepomis macrochirus.

Study: Woodward Research Corporation. No date. Simazine, acute toxicity in sunfish. EPA MRID 23321.

LC₅₀ exceeds 2S so study automatically rates N and cannot be used for criteria derivation.

Lepomis macrochirus.

Study: Swabey YH and Schnk CF. (1963) Report on algicides and aquatic herbicides. Ontario Water Resources Commission. EPA MRID 34214.

Low chemical purity (50 %). LC₅₀ was not determined because chemical was non-toxic at all tested concentrations. Highest concentration tested (18,000 μ g/L) exceeded 2S. Study automatically rates N and cannot be used in criteria derivation.

Oncorhynchus mykiss

Study: Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates*. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.

RelevanceReliabilityScore: 60Score: 54.5Rating: NRating: N

Relevance points taken off for: Standard method (10), Controls (15), Point estimates (15). 100-40=60

	Johnson & Finley 1980	O. mykiss
Parameter	Value	Comment
Test method cited	Not reported	
Phylum	Chordata	
Class	Actinopterygii	
Order	Salmoniformes	
Family	Salmonidae	
Genus	Oncorhynchus	
Species	mykiss	
Family native to North America?	Yes	
Age/size at start of test/growth	1.2 g	
phase		
Source of organisms	Federal or State hatchery	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	Not reported	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	12 ± 1 °C	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Reconstituted deionized	
	water	
pН	7.2-7.5	
Hardness	40-50 mg/L CaCO ₃	
Alkalinity	30-35 mg/L CaCO ₃	
Conductivity	Not reported	
Dissolved Oxygen	Not reported	Aerated beforehand

	Johnson & Finley 1980	O. mykiss
Parameter	Value	Comment
Feeding	Not fed	
Purity of test substance	98.1 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	≤0.5 mL/L acetone	
test solutions		
Concentration 1 Nom; Meas (µg/L)	≥6 concentrations tested but	2 reps, 10/rep
	not reported	
Control	Not reported	
LC ₅₀ (95% CI) (μg/L)	>100	Method: Litchfield
		and Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation</u>: Control type (8), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8), Statistical significance (2), Significance level (2), Minimum significant difference (2), % control at NOEC/LOEC (2), Point estimates (8). Total: 100-43 =57

Acceptability: Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), Point estimates (3). Total: 100-48 = 52

Reliability score: mean(57,52)=54.5

Oncorhynchus mykiss.

Study: Kuc WJ. (1976) Acute toxicity of simazine technical, batch # FL-750336, to the rainbow trout, *Salmo gairdneri*. Aquatic Environmental Sciences, Tarrytown, New York. USEPA MRID 43666.

LC₅₀ exceeds 2S so study rates N and cannot be used in criteria derivation.

Pteronarcys californica

Study: Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates*. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.

RelevanceReliabilityScore: 75Score: 60Rating: LRating: L

Relevance points taken off for: Standard method (10), Controls (15). 100-25 = 75

	Johnson & Finley 1980	P. californica
Parameter	Value	Comment
Test method cited	Not reported	
Phylum	Arthropoda	
Class	Insecta	
Order	Plecoptera	
Family	Pteronarcyidae	
Genus	Pteronarcys	
Species	californica	
Family native to North America?	Yes	
Age/size at start of test/growth phase	Second year class	
Source of organisms	Invertebrates collected from wild and cultured in laboratory	
Have organisms been exposed to contaminants?	No	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	48 h	
Data for multiple times?	Not reported	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	15 ± 1 °C	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Reconstituted deionized	
	water	
pH	7.2-7.5	
Hardness	40-50 mg/L CaCO ₃	
Alkalinity	30-35 mg/L CaCO ₃	
Conductivity	Not reported	

	Johnson & Finley 1980	P. californica
Parameter	Value	Comment
Dissolved Oxygen	Not reported	Aerated beforehand
Feeding	Not fed	
Purity of test substance	98.1 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	≤0.5 mL/L acetone	
test solutions		
Concentration 1 Nom; Meas (µg/L)	≥6 concentrations tested but	2 reps, 10/rep
, ,	not reported	
Control	Not reported	
EC ₅₀ (95% CI) (μg/L)	1900 (900-4000)	Method: Litchfield
		and Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation</u>: Control type (8), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8), Statistical significance (2), Significance level (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-35 =65

<u>Acceptability:</u> Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1). Total: 100-45 =55

Reliability score: mean(65,55)=60

Pacifastacus leniusculus

Study: Velisek, J. 2013. Acute toxicity of triazine pesticides to juvenile signal crayfish (*Pacifastacus leniusculus*). Neuroendrocrinology Letters. 34: 31-36.

All LC₅₀ values (48 h: 206,300; 72 h: 58,700; 96 h: 30,600) exceed 2S (10, 900 μ g/L) so study rates N and cannot be used in criteria derivation.

Pimephales promelas

Study: Johnson WW and Finley MT. (1980) U.S. Department of Interior, Fish and Wildlife Service. *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates*. Resource Publication No. 137. Washington, DC: U.S. Government Printing Office.

RelevanceReliabilityScore: 60Score: 54.2Rating: NRating: N

Relevance points taken off for: Standard method (10), Controls (15), Point estimates (15). 100-40=60

	Johnson & Finley 1980	P. promelas
Parameter	Value	Comment
Test method cited	Not reported	
Phylum/subphylum	Chordata	
Class	Actinopterygii	
Order	Cypriniformes	
Family	Cyprinidae	
Genus	Pimephales	
Species	promelas	
Family native to North America?	Yes	
Age/size at start of test/growth	0.7 g	
phase		
Source of organisms	Hatchery	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	Yes	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	Not reported	
Effect 1	Immobilization	
Control response 1	Not reported	
Temperature	25 ± 1 °C	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Reconstituted deionized	
	water	
pН	7.2-7.5	
Hardness	40-50 mg/L CaCO ₃	
Alkalinity	30-35 mg/L CaCO ₃	
Conductivity	Not reported	
Dissolved Oxygen	Not reported	Aerated beforehand

	Johnson & Finley 1980	P. promelas
Parameter	Value	Comment
Feeding	Not fed	
Purity of test substance	98.1 %	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Not reported	
nominal or measured		
concentrations?		
Chemical method documented?	Not reported	
Concentration of carrier (if any) in	≤0.5 mL/L acetone	
test solutions		
Concentration 1 Nom; Meas (µg/L)	≥6 concentrations tested but	2 reps, 10/rep
	not reported	
Control	Not reported	
LC ₅₀ (95% CI) (μg/L)	>100	Method: Litchfield
		and Wilcoxon

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation</u>: Control type (8), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Dissolved oxygen (4), Conductivity (2), Photoperiod (3), Hypothesis tests (8), Statistical significance (2), Significance level (2), Minimum significant difference (2), % control at NOEC/LOEC (2), Point estimates (8). Total: 100-43 =57

Acceptability: Standard method (5), Appropriate control (6), Control response (9), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Organisms randomized (1), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Random design (2), Dilution factor (2), Minimum significant difference (1), % control at NOEC (1), % control at LOEC (1), Point estimates (3). Total: 100-48 =52

Reliability score: mean(57,52)=54.2

Pimephales promelas

Study: Sleight BH. (1971) Acute toxicity of some Ciba-Geigy experimental chemicals to fathead minnows (*Pimephales promelas*). Bionomics, Inc., Wareham, Massachusetts. USEPA MRID 33309.

RelevanceReliabilityScore: 70Score: 48Rating: LRating: N

Relevance points taken off for: Chemical purity (15), Controls (15). 100-30=70

	Sleight 1971	P. promelas
Parameter	Value	Comment
Test method cited	Fish-Pesticide Acute	
	Toxicity Test Method	
	(USDA), Fish Bioassay	
	Procedure (USPHA, 1970)	
Phylum/subphylum	Chordata	
Class	Actinopterygii	
Order	Cypriniformes	
Family	Cyprinidae	
Genus	Pimephales	
Species	promelas	
Family native to North America?	Yes	
Age/size at start of test/growth	1.5 g, 58 mm	
phase		
Source of organisms	Commercial hatchery in	
	Arkansas	
Have organisms been exposed to	No	
contaminants?		
Animals acclimated and disease-	24 h	
free?		
Animals randomized?	Not reported	
Test vessels randomized?	Not reported	
Test duration	96 h	
Data for multiple times?	24, 96 h	
Effect 1	Mortality	
Control response 1		
Temperature	$18 \pm 0.5 {}^{\circ}\text{C}$	
Test type	Static	
Photoperiod/light intensity	Not reported	
Dilution water	Reconstituted deionized	
	water	
pH	7.1	
Hardness	Not reported	

	Sleight 1971	P. promelas
Parameter	Value	Comment
Alkalinity	35 mg/L CaCO ₃	
Conductivity	Not reported	
Dissolved Oxygen	8.1-5.6 mg/L	Not aerated; 85-59 %
Feeding	Not reported	
Purity of test substance	Not reported	
Concentrations measured?	Not reported	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on nominal or measured concentrations?	Not reported	
Chemical method documented?	Not reported	
Concentration of carrier (if any) in test solutions	Acetone, concentration not reported	
Concentration 1 Nom; Meas (µg/L)	Concentrations not reported	Replicates not reported
Control	Not reported	
LC ₅₀ (95% CI) (μg/L)	24 h: 16,5000 (8,200- 24,000) > 2S 96 h: 6,400 (4,800-8,700)	Method: probit
NOEC	2,500	Method: Linear regression p: Not reported MSD: Not reported
% control at NOEC	Not calculable	

Notes: Data not included in report.

Simazine solubility (S) = $5,450 \mu g/L$, $2S = 10,900 \mu g/L$.

Reliability points taken off for:

<u>Documentation:</u> Control type (8), Chemical purity (5), Analytical method (4), Nominal concentrations (3), Measured concentrations (3), Hardness (2), Conductivity (2), Photoperiod (3), Hypothesis tests (8). Total: 100-38 =62

Acceptability: Appropriate control (6), Control response (9), Chemical purity (10), Measured concentrations within 20% nominal (4), Concentrations not > 2x solubility (4), Carrier solvent (4), Organisms randomized (1), Adequate organisms per rep (2), Feeding (3), Hardness (2), Dissolved oxygen (6), Conductivity (1), Photoperiod (2), Number of concentrations (3), Random design (2), Adequate replication (2), Dilution factor (2), Hypothesis tests (3). Total: 100-66 = 34

Reliability score: mean(62,34)=48

Pimephales promelas

Study: Bionomics 1971. Acute toxicity of some Ciba-Geigy experimental chemicals to fathead minnow (*Pimephales promelas*). Bionomics, Inc., Wareham Massachusetts. Submitted to Ciba-Geigy Corporation, Greensboro, North Carolina. EPA MRID 31150.

LC50 exceeds 2S so study automatically rates N and cannot be used for criteria derivation.

Vallisneria americana Michx

Study: Wilson, P.C. and Wilson, S.B., 2010. Toxicity of the herbicides bromacil and simazine to the aquatic macrophyte, Vallisneria americana Michx. Environmental Toxicology and Chemistry, 29(1), 201-211.

RelevanceReliabilityScore: 67.5Score: 70Rating: NRating: L

Relevance points taken off for: Standard (10), Chemical purity (15), Control response (7.5). 100-17.5=82.5

	Wilson & Wilson 2010	V. americana Michx
Parameter	Value	Comment
Test method cited	Not reported	
Order	Alismatales	
Family	Hydrocharitaceae	
Genus	Vallisneria	
Species	Americana	
Family native to North America?	Yes	
Age/size at start of test/growth phase	0.22-1.97 g	
Source of organisms	Parent plants taken from	
_	Lake Okeechobee, Florida	
Have organisms been exposed to contaminants?	Not reported	
Animals acclimated and disease-free?	Yes	
Animals randomized?	Yes	
Test vessels randomized?	Yes	
Test duration	13 d	
Data for multiple times?	13 d exposure	
	27 d exposure+recovery period	
Effect 1	Fresh weight gain	
Control response 1	Not reported	
Effect 2	Reproduction	Number daughter plants
Control response 2	Not reported	
Temperature	25 °C	
Test type	Static	Single-pulse
Photoperiod/light intensity	16:8 light:dark; 76 μg mol/s/m ²	
Dilution water	Tap water	Very hard
pH	8.2	

	Wilson & Wilson 2010	V. americana Michx
Parameter	Value	Comment
Hardness	226 mg/L CaCO ₃	
Alkalinity	207 mg/L CaCO ₃	
Conductivity	826 μS	
Dissolved Oxygen	Not reported	
Feeding	Slow release fertilizer,	
	Nutricote 13-13-13	
Purity of test substance	Not reported	
Concentrations measured?	Yes	
Measured is what % of nominal?	Not reported	
Toxicity values calculated based on	Measured	
nominal or measured		
concentrations?		
Chemical method documented?	GC	
Concentration of carrier (if any) in	Not reported	
test solutions		
Concentration 1 Nom; Meas (µg/L)	Not reported; 58	5 reps, 1/rep
Concentration 2 Nom; Meas (µg/L)	Not reported; 116	
Concentration 3 Nom; Meas (µg/L)	Not reported; 229	
Concentration 4 Nom; Meas (µg/L)	Not reported; 344	
Concentration 5 Nom; Meas (µg/L)	Not reported; 457	
Concentration 6 Nom; Meas (µg/L)	Not reported; 592	
Control	0; 0	
EC ₅₀ (95% CI) (μg/L)	Growth:	Method: ANOVA
	13 d: 67	
	27 d (exposure+recovery):	CI not reported
	86	
	Reproduction: 144	
NOEC	<58	Method: ANOVA
		p: 0.05
		MSD: Not reported
LOEC	58	
MATC (GeoMean NOEC, LOEC)	Not calculable	
% control at NOEC	Not calculable	
% control at LOEC	Not calculable	

Simazine solubility (S) = 5,450 μ g/L, 2S = 10, 900 μ g/L.

Reliability points taken off for:

<u>Documentation:</u> Chemical purity (5), Nominal concentrations (3), Dissolved oxygen (4), Statistical significance (2), Minimum significant difference (2), % control at NOEC/LOEC (2). Total: 100-18 = 82

<u>Acceptability:</u> Chemical purity (10), Standard method (5), Control response (9), Measured concentrations within 20% nominal (4), No prior contamination (4), Dissolved oxygen (6), Temperature variation (3), Hypothesis tests (3). Total: 100-42 =58

Reliability score: mean(82,58)=70